

**Investigating the dynamic process of expressing  
breastmilk for very preterm infants and the  
influence of a relaxation intervention, with the aim  
of improving clinical recommendations**

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## **Abstract**

**Background:** Very preterm infants (born at less than 32 weeks of pregnancy) have higher morbidity and mortality than more mature infants. Even small increases in mother's own milk quantity are associated with medium and long-term impacts on morbidity. Due to the infant's oral immaturity, the mother must extract milk from the breasts (expressing), which is very challenging. Clinical recommendations to express 8-10 times per day are demanding and adherence is low. Relaxation has been identified as a promising intervention for lactation outcomes.

**Aim:** To investigate determinants of the quantity of breastmilk expressed by mothers of very preterm infants, focusing on relaxation interventions and expressing pattern.

### **Methods:**

- 1) Systematic review and meta-analysis of relaxation interventions and milk yield.
- 2) A multi-centre, randomised, controlled trial. Mothers giving birth between 23+0 and 31+6 weeks of pregnancy were recruited. The intervention was a 12-minute guided relaxation recording. The primary outcome was the highest 24-hour milk quantity recorded at day 4, 14 or 21 after birth.
- 3) Exploratory analysis of expressing pattern and lactation outcomes.

**Findings:**

- 1) The systematic review included 1982 participants in 18 studies with heterogeneous relaxation interventions. There was moderate certainty evidence that relaxation increased breastmilk quantity by a moderate amount.
- 2) The randomised trial recruited the target 132 participants. Mean gestational age at birth was 27.8 weeks (SD 2.4). Adjusted mean difference in the primary outcome was 73.9g (95% CI –61.7 to 209.5). The majority of participants liked the intervention and found it relaxing. There were trends within the trial (sub-group analysis) and between trials (meta-regression) suggesting lower effect of relaxation on milk quantity with lower gestation at birth.
- 3) Less than a quarter of participants expressed eight or more times per day. Expressing frequency was positively associated with 24-hour milk yield (94.4g adjusted increase per session at three weeks after birth, 95% CI 62.7 to 126.2). Expressing eight or more times per day was associated with higher milk yield than expressing less than six times per day, but not compared to 6-7 times per day.

**Conclusions:** A weak recommendation can be made to offer relaxation interventions after very preterm birth. Many people value relaxation in this setting and there may be a small increase in milk quantity, which could be clinically important. Evidence for the recommendation to express 8-10 times per day is relatively weak, and this is difficult to achieve. These results can help improve individualised counselling for families.

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Finally, this thesis is dedicated to all the families who have been through neonatal care, particularly the 132 families who gave so much of their time to enrol in this trial at a period of immense stress.



## **Statement of contribution to work**

I, Ilana Levene, confirm that the work presented in this thesis is my own. I conceptualised the research, obtained the funding, gained the required approvals, led the trial setup process, trained and supported all local site staff, recruited the majority of the participants, conducted all the analyses and wrote the thesis. I am first author on two publications<sup>1,2</sup> arising from the work described in Chapters 4 & 5 and on two submitted manuscripts from the work described in Chapters 3 & 6. Professor Maria Quigley, Professor Mary Fewtrell and Dr Frances O'Brien supervised the research from its inception; advising on study design, trial management, statistical analysis and interpretation of results. Pollyanna Hardy joined the supervision team after the start of the clinical trial and provided particular expertise on trials methodology and statistical analysis. All supervisors provided feedback on the thesis. As well as my supervisors, the following colleagues are authors on the published and submitted papers, supporting the work presented in each paper in the planning stages and advising on interpretation and manuscript preparation and final approval: Professor Fiona Alderdice, Beth McCleverty, Jennifer Bell, Christina Cole, Kayleigh Stanbury and Dr Nurul Husna Mohd Shukri. Additionally, Dr Mohd Shukri independently screened abstracts and full text reports and independently made Risk of Bias assessments for the systematic review presented in Chapter 5 and submitted for publication. The co-authors of the published and submitted publications have all given their permission to include all relevant material in this thesis.

## **Inclusivity Statement**

This dissertation will use female-coded language such as 'mothers' to refer to individuals who have given birth and are lactating. However, I recognise and respect that some people who give birth and lactate do not identify as women and that some people are mothers but have not given birth and/or cannot lactate. The participants in the work presented were not asked their gender identity; in future work I will seek this information to ensure that descriptions of trial participants respect and reflect individuals' own identities.

## List of publications

### ***Peer-reviewed publications arising from thesis research:***

**Levene, I.,** Alderdice, F., McCleverty, B., O'Brien, F., Fewtrell, M., & Quigley, M. A. (2022). A report on parent involvement in planning a randomised controlled trial in neonatology and lactation – insights for current and future research. *International Breastfeeding Journal*, 17(1), 69.

**Levene, I.,** Bell, J. L., Cole, C., Stanbury, K., O'Brien, F., Fewtrell, M., & Quigley, M. A. (2022). Comparing the effect of a lactation-specific relaxation and visualisation intervention versus standard care on lactation and mental health outcomes in mothers of very premature infants (the EXPRESS trial): study protocol for a multi-centre, unmasked, randomised, parallel-group trial. *Trials*, 23(1), 611.

**Levene, I.,** Mohd Shukri, N. H., O'Brien, F., Quigley, M. A. & Fewtrell, M. (2024). The impact of relaxation therapy on human milk feeding outcomes. A Meta-analysis. *JAMA Pediatrics*, accepted for publication. DOI 10.1001/jamapediatrics.2024.0814

**Levene, I.,** Quigley, M. A., Fewtrell, M., & O'Brien, F. (2024). Does extremely early expression of colostrum after very preterm birth improve mother's own milk quantity? A cohort study. *Archives of Disease in Childhood (Fetal & Neonatal)*, accepted for publication. DOI 10.1136/archdischild-2023-326784

### ***Submitted publications arising from thesis research (under review at time of final thesis submission):***

**Levene, I.,** Hardy, P., Bell, J. L., Cole, C., Stanbury, K., O'Brien, F., Fewtrell, M., & Quigley, M. A. Audio relaxation versus standard care for lactating parents of very preterm infants (the EXPRESS trial): a randomised controlled trial.

**Levene, I.,** O'Brien, F., Fewtrell, M, & Quigley, M. A. The relationship of early expressed milk quantity and later exclusive breastmilk feeding after very premature birth: a cohort study

**Levene, I.,** Fewtrell, M., Quigley, M. A., & O'Brien, F. (2024). The relationship of milk expression pattern and lactation outcomes after very premature birth: A cohort study

***Other publications published during the period of the DPhil***

Quigley, M. A., Harrison, S., **Levene, I.,** McLeish, J., Buchanan, P., & Alderdice, F. (2023). Breastfeeding rates in England during the Covid-19 pandemic and the previous decade: Analysis of national surveys and routine data. ***PLOS ONE***, 18(10), e0291907.

**Levene, I.,** Adams, E. (2023). The interaction of early exclusive mother's milk feeding and ethnic background with ultimate feeding outcomes after very preterm birth. ***Breastfeeding Medicine***, 18(11), 842-848

**Levene, I.,** Dhami, A., Moreno, M., Shine, B., Chinoy, A., Padidela, R., & Molnar, Z. (2023). Characterisation of parathyroid hormone concentration in extremely preterm or very low birthweight infants in routine clinical screening for metabolic bone disease: A service evaluation cohort study. ***Journal of Paediatrics and Child Health***, 59(10).

## Abbreviations

|              |                                                                                 |
|--------------|---------------------------------------------------------------------------------|
| ACTH         | Adrenocorticotrophic Hormone                                                    |
| AMED         | Allied and Complementary Medicine Database                                      |
| BAI          | Beck Anxiety Index                                                              |
| BFI          | Baby Friendly Initiative                                                        |
| BSES-SF-NICU | Breastfeeding Self-Efficacy Scale – Short Form - NICU                           |
| CA           | Corrected Age                                                                   |
| CF           | Complementary Food                                                              |
| CI           | Confidence Interval                                                             |
| CINAHL       | Cumulative Index to Nursing and Allied Health Literature                        |
| CRF          | Case Report Form                                                                |
| CTU          | Clinical Trials Unit                                                            |
| DASS         | Depression, Anxiety and Stress Subscale                                         |
| DMC          | Data Monitoring Committee                                                       |
| DS           | Domain S                                                                        |
| DSM-IV       | Diagnostic and Statistical Manual of Mental Disorders – 4 <sup>th</sup> Edition |
| DSM-5        | Diagnostic and Statistical Manual of Mental Disorders – 5 <sup>th</sup> Edition |
| EDD          | Expected Date of Delivery                                                       |
| EFSA         | European Food Safety Authority                                                  |
| ELISA        | Enzyme-linked Immunosorbent Assay                                               |
| EPDS         | Edinburgh Postnatal Depression Scale                                            |
| ESPGHAN      | European Society for Paediatric Gastroenterology, Hepatology, and Nutrition     |
| EXPRESS      | EXpressing in PREmaturity – Simple interventionS (RCT title acronym)            |
| GDG          | Guideline Development Group                                                     |
| GRADE        | Grading of Recommendations, Assessment, Development and Evaluation              |
| IES          | Impact of Event Scale                                                           |
| IMD          | Index of Multiple Deprivation                                                   |
| IQR          | Interquartile Range                                                             |
| MD           | Mean Difference                                                                 |
| Med Diff     | Median Difference                                                               |
| MER          | Milk Ejection Reflex                                                            |
| MOM          | Mother's Own Milk                                                               |
| MP3          | Motion Pictures Expert Group Audio Layer III (digital audio file)               |
| NA           | Not Applicable                                                                  |
| NEC          | Necrotising Enterocolitis                                                       |
| NICE         | National Institute for Health and Care Excellence                               |
| NICU         | Neonatal Intensive Care Unit                                                    |
| NIHR         | National Institute for Health and Social Care Research                          |
| NNAP         | National Neonatal Audit Programme                                               |
| NPEU         | National Perinatal Epidemiology Unit                                            |
| OR           | Odds Ratio                                                                      |
| PASS         | Perinatal Anxiety Screening Scale                                               |
| PCL-5        | Post-traumatic Stress Checklist for DSM-5                                       |
| PICO         | Population-Intervention-Comparison-Outcome                                      |
| PIS          | Patient Information Sheet                                                       |
| PMA          | Post Menstrual Age                                                              |

|            |                                                                    |
|------------|--------------------------------------------------------------------|
| PMG        | Project Management Group                                           |
| PPEET      | Public and Patient Engagement Evaluation Tool                      |
| PPI        | Patient and Public Involvement                                     |
| PPIE       | Patient and Public Involvement and Engagement                      |
| PRISMA     | Preferred Reporting Items for Systematic Reviews and Meta-Analyses |
| PSS        | Perceived Stress Scale                                             |
| PTS        | Post-Traumatic Stress                                              |
| PTSD       | Post-Traumatic Stress Disorder                                     |
| RCT        | Randomised Controlled Trial                                        |
| ROB        | Risk of Bias                                                       |
| ROP        | Retinopathy of Prematurity                                         |
| RR         | Relative Risk                                                      |
| SAP        | Statistical Analysis Plan                                          |
| SARS-CoV-2 | Severe Acute Respiratory Syndrome Coronavirus 2                    |
| SCL-90-R   | Symptom Check List (90-R)                                          |
| SD         | Standard Deviation                                                 |
| SDS        | Standard Deviation Score                                           |
| SMD        | Standardised Mean Difference                                       |
| SMS        | Short Message Service                                              |
| STAI       | Spielberger State Trait Anxiety Index                              |
| TSC        | Trial Steering Committee                                           |
| UK         | United Kingdom                                                     |
| UNICEF     | United Nations Children's Fund                                     |
| URL        | Uniform Resource Locator                                           |
| US         | United States                                                      |
| USA        | United States of America                                           |
| VAS        | Visual Assessment Scale                                            |
| VLBW       | Very Low Birth Weight                                              |
| WHO        | World Health Organisation                                          |

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# Chapter 1: Thesis Overview

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## 1.2 Introduction

An estimated 14.9 million infants are born preterm (at less than 37 weeks of pregnancy) globally each year, of whom 2.4 million are born very preterm (at less than 32 weeks of pregnancy)<sup>3</sup>. Mothers' own breastmilk is particularly important for very preterm infants, reducing morbidity and mortality<sup>4–9</sup>. Providing breastmilk can also be a source of psychological connection and repair for mothers after the trauma of very preterm birth<sup>10–16</sup>. Milk must be extracted from the breasts (expressed) to give to the infant through a tube directly into the stomach due to their immaturity. However, many mothers find it difficult to express sufficient milk for their infant or achieve their breastmilk feeding goals<sup>17–22</sup>, which causes anxiety and distress<sup>23</sup>. The consensus recommendations for how to optimally express<sup>24</sup> milk are burdensome and difficult for families<sup>23,25–28</sup>, competing with time that the mothers would like to spend with their infant or on self-care<sup>29–31</sup>, including sleep. Adherence to these recommendations is low<sup>32–34</sup>.

Although improving breastmilk feeding after preterm birth was identified as a top ten research priority by the James Lind Alliance<sup>35</sup>, there are few randomised controlled trials relating to breastmilk expression<sup>32</sup>. Relaxation therapy was identified by a Cochrane review as a promising intervention for further randomised evaluation<sup>32</sup>.

The predominantly observational literature investigating optimal dynamics of milk expression includes many studies with small samples sizes (fewer than 50 participants<sup>36–46</sup>) and has produced results that are difficult to apply clinically. There is therefore a large unmet need for clinically-focused research on breastmilk expression, with the potential to increase the quantity and duration of breastmilk provision for these vulnerable infants, as well as improve maternal experience and mental health.

### 1.3 Thesis Aims & Objectives

This thesis aims to investigate determinants of the quantity of breastmilk that can be expressed by mothers of very preterm infants, and related breastfeeding outcomes, with particular focus on relaxation interventions and the pattern of expressing. The specific objectives are:

1. To review current evidence on the effect of relaxation therapy on lactation and mental health outcomes in lactating mothers and their infants.
2. To investigate the effect of relaxation therapy on breastmilk yield, breastfeeding duration and mental health outcomes in mothers of very preterm infants, using a randomised controlled design.
3. To use the cohort constructed for the randomised trial in order to explore the association of expressing pattern (frequency, timing and gaps) and lactation outcomes.
4. To explore how the insights gained can be used in clinical practice and set hypotheses for future research.

### 1.4 Thesis structure

This thesis is presented in seven further chapters:

**Chapter 2: Introduction.** This chapter provides an introduction to prematurity, the impact of breastmilk, the difficulties experienced by mothers in attempting to express and maternal mental health after very preterm birth. It explores the connections between lactation and mental health, then outlines the nature of relaxation interventions and the theoretical pathways through which they could affect lactation outcomes. It concludes with an overview of the impact of current clinical recommendations on expressing pattern on families. This chapter sets the scene for all the thesis objectives.

**Chapter 3: The effect of relaxation interventions on lactation: a systematic review update with meta-analysis.** This chapter systematically reviews the literature on the impact of relaxation interventions on lactation, mental health and related outcomes for lactating mothers and infants.

Detailed meta-analysis is presented and the impact of the findings on the experimental study design are discussed. This chapter addresses research objective 1.

**Chapter 4: Parent Involvement to design a randomised controlled trial of relaxation after very preterm birth.** This chapter describes the iterative process of consultation with parents of preterm infants that took place in order to plan, design and carry out the experimental work. This is an example of Public Patient Involvement in research (PPI). The influence of each stage of PPI on the experimental work and exploratory questions is discussed. This chapter informs research objective 2 and 3.

**Chapter 5: Study methods for a randomised controlled trial of relaxation after very preterm birth.** This chapter presents the experimental methods used to carry out a randomised controlled trial of a relaxation intervention for parents of very preterm infants. Ethical issues are discussed and inherent risks of bias implicit in the study design are presented, with mitigation strategies. This chapter provides the methods used to address research objective 2.

**Chapter 6: Results of a randomised controlled trial of relaxation after very preterm birth.** This chapter presents the results of the randomised controlled trial, starting with the baseline characteristics of the study participants. Primary outcome data is presented, with sensitivity analyses and subgroup analyses. Subsequently, secondary outcomes and process indicators are presented. Key outcomes of the meta-analysis presented in Chapter 3 are re-visited to integrate the results of the randomised trial and the initial importance of the results is discussed. This chapter addresses research objective 2.

**Chapter 7: Exploratory analysis of the pattern of milk expression.** This chapter introduces the literature informing current clinical recommendations for parents on how to best express milk, in terms of frequency, timing and gaps between sessions. It then defines the pre-specified exploratory questions and statistical analysis plan to be used with the cohort data arising from the randomised trial. The results of the exploratory work are presented and their initial importance discussed. This chapter addresses research objective 3.

**Chapter 8: Overall discussion, conclusion and clinical recommendations.** This chapter explores the strengths and limitations of the work presented, its place within the wider literature and proposes hypotheses for future research. The impact on clinical guidance is discussed in detail, with reference to literature on clinical reasoning, health psychology and ethics. An analogous example of guidance on the timing of complementary feeds is used to support these arguments. This chapter uses the work described in previous chapters to address research objective 4.

# Chapter 2: Introduction

---

## 2.1 Chapter Overview

The research presented in this thesis explores selected determinants of the quantity of breastmilk that mothers can express after giving birth very prematurely, and their longer-term breastmilk feeding outcomes. This chapter provides the background in relation to the epidemiology of preterm birth, the value of mothers' own milk in this setting, the difficulties encountered in expressing milk and their connection with maternal mental health. A rationale for the investigation of relaxation interventions on breastmilk outcomes is described in this setting. Finally, qualitative evidence on the burden of current clinical recommendations for milk expression will be presented, along with the context of established influences on lactation outcome in the exclusively expressing setting. Breastmilk feeding is used as a term covering the provision of breastmilk by any route (for example, directly at the breast, by gastric tube or by bottle).

## 2.2 Background

### 2.2.1 Preterm birth and mother's own milk

Full term pregnancy is defined as birth following at least 37 completed weeks from the first day of the last menstrual period (or equivalent using ultrasound dating). Preterm birth can be divided by the length of gestation of the pregnancy into late preterm (34 to 36 weeks of post-menstrual age; PMA), moderately preterm (32 to 33 weeks' PMA), very preterm (28 to 31 weeks' PMA) and extremely preterm (less than 28 weeks' PMA). The rate of preterm birth is increasing<sup>47</sup>, with an estimated 14.9 million infants born preterm globally each year, of which 2.4 million are very or extremely preterm<sup>3</sup>. Complications arising from preterm birth are the leading cause of neonatal death in the UK<sup>48</sup> and globally<sup>47,49</sup>, leading to an estimated 0.9 million deaths worldwide per year. Preterm infants also have increased risk of long-term disability, which increases as the gestational age at birth decreases<sup>50</sup>. Therefore, in addition to the direct healthcare costs of admission, the cost



of preterm birth to the public sector over the first 18 years after birth in the UK is over £3 billion; £62,000 per surviving very preterm infant and £95,000 per surviving extremely preterm infant<sup>51</sup>.

Very preterm infants rarely have the oral skills to directly breast or bottle feed at birth and are instead given nutrition intravenously and/or directly into the stomach with a tube. The ideal form of enteral nutrition for these infants is their mother's breastmilk (alternatives being donor human milk or preterm infant formula). For example, very low birth weight (VLBW) infants who receive less than half their nutrition as mother's milk in the first 10 days of life are 60% more likely to die, have necrotising enterocolitis (NEC; a potentially lethal gut infection) or other serious infection<sup>4</sup>. Providing exclusive mother's milk to only 21 preterm infants, in comparison to any formula, would prevent one case of severe retinopathy of prematurity (ROP, one of the commonest causes of childhood blindness<sup>8</sup>). Increasing the amount of mother's milk provided also incrementally improves brain growth<sup>5</sup> and long-term neurodevelopmental outcomes<sup>5-7</sup>.

Data on the health impact of mother's milk is prone to potential confounding as mother's milk exposure cannot be randomised – and while the studies mentioned here adjust for multiple confounders such as maternal education and age, residual unmeasured confounding cannot be fully excluded. Randomised trials of donor human milk have confirmed a reduction in NEC compared to infant formula, but not a long-term neurodevelopmental benefit<sup>52,53</sup>. Donor human milk is not equivalent to mother's milk due to processing (increased freeze-thaw cycles and pasteurisation), the physical disconnection of the milk donor and the infant, which limits the personalisation of immune factors such as antibodies to specific pathogens in the NICU environment, and due to differences in milk constituents according to gestation and time since birth if the donor has an older infant, born at term. This may explain the disparity in neurodevelopmental impact. Despite the inherent methodological weaknesses in this area of evidence, it is clear that maximising early and continued mother's own milk for each preterm infant has an impact on their chances of survival and their long-term health.

Lack of mother's milk also impacts healthcare costs – VLBW infants in the USA in the lowest tertile of mother's milk intake have 22% higher overall neonatal admission costs (adjusted for other factors) than those in the highest tertile<sup>54</sup>. Increasing any breastmilk for infants at discharge home from 50% to 75% in the UK could lead to annual savings of nearly £4 million from reduced cases of NEC during the admission alone, as estimated in 2012<sup>55</sup>. Non-NEC costs are also significant – for example each additional 1mL/kg/day of mother's milk provided during the first two weeks after birth was associated with lower USA non-NEC hospital costs by \$534 in one study, meaning that increasing mother's milk provision by one standard deviation would be associated with a reduction in non-NEC costs by \$10,000 per infant<sup>9</sup>. Complete elimination of formula for preterm infants in the UK could lead to annual savings of £30 million during the primary (birth) hospital admission or £47 million when including wider healthcare costs. 238 lives could be saved each year through reduced sepsis, NEC and sudden infant death syndrome, associated with a £153 million increase in economic lifetime productivity<sup>56</sup>.

Breastmilk feeding at discharge from hospital and beyond is important because of the general public health benefits for both infant and mother in addition to the benefits specific to prematurity. These general benefits include reduction in sudden infant death syndrome, infections during the period of breastmilk consumption, childhood obesity and maternal cancer<sup>57</sup>.

To provide breastmilk for their infants until they are mature enough to attempt breastfeeding directly, mothers must express milk from their breasts manually (i.e. with their hands or with the assistance of a suction pump). Consensus recommendations from the UNICEF UK Baby Friendly Initiative (BFI) are to start expressing within two hours of birth and to continue expressing 8-10 times per day until the infant can breastfeed directly<sup>24</sup>; for infants born at the most preterm gestations this can be for several months. Despite motivation to provide breastmilk for their infants, there is a high risk of poor milk supply, leading to non-exclusive breastmilk feeding<sup>18-21,58</sup> and an increasing failure to meet mothers' own goals for breastmilk provision as time goes on<sup>17</sup>. Low milk supply is a key driver for cessation of expressing and breastfeeding<sup>30,34</sup>, whereas confidence in milk

supply motivates mothers to continue<sup>11</sup>. Of the very preterm infants born in the UK in 2020, 40% were exclusively formula fed at discharge home, and this figure has not improved in the last seven years<sup>59</sup>.

It is challenging to establish and then maintain a full milk supply through exclusive expressing<sup>23,25,60</sup>, which is exacerbated when pregnancy has been abbreviated and the breast tissue<sup>61</sup> and hormonal milieu are therefore not at an optimal stage. Perinatal interventions associated with preterm birth may also pose lactation challenges – such as provision of antenatal steroids, which can delay milk ‘coming in’<sup>62</sup> (‘lactogenesis II’ or ‘secretory activation’, the process of increasing volume and change in consistency from colostrum to mature milk), and caesarean birth. Factors predisposing to preterm birth may also impair lactation, such as smoking<sup>47,63</sup>.

Demographic characteristics associated with poorer breastfeeding outcomes are associated with increased risk of preterm birth, such as teenage pregnancy and socioeconomic disadvantage<sup>47,64</sup>. Countries with higher background breastfeeding rates also have higher rates of long-term breastfeeding after very preterm birth compared to countries with lower background breastfeeding rates<sup>65</sup>. However, long-term breastfeeding rates remain lower for very preterm infants compared to those born full term even in high breastfeeding countries<sup>19</sup>, implying that the poor breastfeeding outcomes seen in the UK very preterm population are likely to comprise both general sociocultural factors and factors specific to prematurity.

The World Health Organisation has included “how can exclusive breastfeeding be promoted, supported and scaled up for preterm or low birth weight infants, especially those who are very preterm or very low birth weight?” as a high priority research question in the area of preterm birth<sup>66</sup>. In the UK, the James Lind Alliance priority setting partnership has identified ‘What type of support is most effective at improving breastfeeding for preterm infants?’ in the top 10 priorities for research in preterm birth<sup>35</sup>. In addition, understanding how to optimise milk synthesis and removal in pump dependent mothers of preterm infants has been identified as a critical unresolved

question for future research by a researcher-led international consensus process within the field of lactation<sup>60</sup>.

### **2.2.2 Maternal mental health after very preterm birth, and its connection to lactation**

Preterm birth has a significant effect on parental mental health. A recent systematic review including over 6,000 parents estimated that 42% had significant anxiety and 40% had significant post-traumatic stress reactions in the first month after birth when the infant had been admitted for neonatal care, with a greater impact on mothers compared to fathers<sup>67</sup>. Between 29% and 40% of mothers of preterm infants had depression in this time period<sup>68</sup>. These rates are higher than the background prevalence of depression and anxiety after term, healthy birth<sup>67-69</sup>. Preterm birth is a risk factor for postnatal anxiety and depression, along with related phenomena such as fear of infant death during delivery<sup>70</sup>. For example in a large USA cohort (n = 4,451), extremely preterm birth was associated with adjusted odds ratio of 2.0 for anxiety and 5.7 for combined anxiety and depression, compared to term birth<sup>71</sup>.

Literature from qualitative studies note key themes related to distress after an infant's neonatal unit admission, including inability to protect the infant from pain, loss of control, helplessness, fear for the infant's outcome, guilt over perceived responsibility for birth events, disconnection from the infant, fear to touch the infant because of their fragility and a loss of the parenting role<sup>72-74</sup>. Parents report a state of continuous alertness because of the ongoing risk of health deterioration or death<sup>26,29</sup>.

A need for closeness and connection is important for parents to repair their sense of having a parental role in this context<sup>72,74</sup>. However it is difficult to create a sense of intimacy with their infant in the surroundings of the neonatal intensive care unit, which is filled with staff, other parents and negative sensory experiences such as alarm bells; with little privacy and where physical contact with the infant is sometimes limited by healthcare professionals<sup>26,29,75</sup>. The physical trauma of preterm birth is a further stressor as mothers may be in pain, anaemic and exhausted<sup>29</sup>.

Maternal depression and anxiety is associated with poorer breastmilk feeding outcomes in general, as well as in the specific setting of prematurity<sup>76</sup>. It is difficult to disentangle the directionality of this relationship, and there are likely to be bidirectional effects (something that is raised by mothers themselves<sup>11</sup>). For example, in support of a 'poor mental health impairs lactation' hypothesis, negative mood during pregnancy is associated with increased use of infant formula in large Norwegian cohort studies<sup>77</sup>. The effect of antenatal anxiety and depression is persistent after adjusting for postnatal mood and other perinatal factors<sup>78</sup>.

Mothers report that anxiety over their infant's condition is a barrier to breastfeeding<sup>34,73</sup>, that stress is a reason to cease lactation by six weeks after birth<sup>30</sup> and that anxiety over milk volumes can lead to behavioural changes such as cessation of expressing<sup>12</sup>. It has been hypothesised that maternal distress could impair lactation capability, either through hormonal pathways<sup>79</sup>, neuronal pathways or behavioural signalling<sup>80</sup> between mother and infant. For example, markers of physical stress in labour such as umbilical cord glucose, maternal exhaustion score and length of labour are associated with delayed secretory activation (lactogenesis II)<sup>81</sup>, as are cortisol levels<sup>82</sup>. Imposing stress on breastfeeding women through mental calculation or noise impairs the pulsatile release of the lactogenic hormone oxytocin during suckling<sup>83</sup>. Activation of the sympathetic-adrenal axis (associated with "fight or flight" stress) acts centrally and peripherally to inhibit milk ejection<sup>84</sup>. Maternal depression is associated with lower oxytocin even before birth<sup>85</sup>.

In support of the alternative 'poor lactation impairs mental health' hypothesis, inability to meet mothers' breastmilk feeding goals increases the risk of postnatal depression in comparison to both meeting breastmilk feeding goals and having no intention to breastmilk feed<sup>86</sup>. Difficulties with lactation are reported to cause unhappiness and guilt<sup>75</sup>, as outlined in the extracts from a Swedish qualitative study below. Mothers also report positive lactation experiences causing them to feel satisfied and happy<sup>11</sup>.

*"...she didn't have the energy [to breastfeed], she was too little, so she didn't manage to suck and that was that, that was just another sorrow that one had to get through"* (participant 8, Morelius et al 2020)<sup>75</sup>

*"Because I gave up, I felt as a bad mother who couldn't manage to get it going"* (participant 7, Morelius et al 2020)<sup>75</sup>

When trying to separate out the directionality of the relationship of anxiety and breastfeeding cessation, one very large Norwegian cohort study including mothers giving birth at all gestations (n = 42,225) suggested that about half of the association appears to be related to pre-existing anxiety increasing the likelihood of stopping breastfeeding and about half to stopping breastfeeding increasing anxiety. There is also an interaction effect, where those who have higher levels of pre-existing anxiety experience a greater increase in anxiety after breastfeeding cessation<sup>78</sup>. A final variable increasing the complexity of assessing these associations is that antidepressant medication (selective serotonin reuptake inhibitors) may directly impair secretory activation<sup>87</sup>.

Literature from qualitative studies emphasises that anxiety over expressing milk is common and a major source of distress for mothers of very preterm infants<sup>23,25-28</sup>, along with the significant physical burden of expressing milk so frequently, night and day. A key theme is that expressing milk can symbolise both disconnection and connection with the infant and is therefore a source of distress and a potential source of psychological repair<sup>10,73,74</sup>.

The disconnection is related to the mechanical and alien nature of the expressing process<sup>15</sup> and the fact that expressing milk reminds the mother of the absence or fragility of the infant<sup>11,88</sup>. The potential for connection relates to the meaning placed on the milk as a gift only the mother can give<sup>11-15,74</sup> ("the umbilical cord outside the uterus"<sup>73</sup>), and the investment in the future potential of breastfeeding when the infant is ready<sup>11,75</sup>. This is described by Nancy Hurst after considering the experiences of the mothers in her USA qualitative study<sup>10</sup>:

*"To reach her ultimate goal to breastfeed, the mother of a very preterm infant often must replace her infant at breast with a mechanical breast pump to stimulate and maintain her milk production. The detachment from the infant that the pump represents, along with the importance of it to provide sustenance to her infant, leads to a paradoxical reaction in the mother. This situation presents the ultimate paradox of the pump as both a link and a wedge between the mother and her infant"*

The theme of psychological repair or balance is often brought up by mothers, as demonstrated in these extracts from qualitative studies in the US, China and Taiwan:

*"It was a way for me to still feel that she was a part of me."* (anonymous participant, Rossman et al 2013)<sup>15</sup>

*"I hope my milk will connect me to my baby"* (participant T, Yang et al 2022)<sup>89</sup>

*"I'm doing for her outside what I couldn't do for her in my womb."* (anonymous participant, Rossman et al 2013)<sup>15</sup>

*"Even if I can't be there for him every single day, I'm pumping still. So you feel like you're doing something for him."* (anonymous participant, Rossman et al 2013)<sup>15</sup>

*"I feel really connected to her and that I'm doing something for her, not just putting something down a tube or changing a nappy, that I'm really providing for her."* (anonymous participant, Swanson et al 2012)<sup>74</sup>

*"It was me who let the preterm birth occur, so I have decided to feed him breast milk. It is a sense of compensation for me."* (anonymous participant, Lee et al 2009)<sup>16</sup>

It is therefore unsurprising that mothers often value ways in which they can incorporate elements of connection into the expressing process, for example by expressing at the cot side or when seeing and smelling items that remind them of the infant<sup>11,16,74,75</sup>. However, this can be extremely difficult, particularly when direct contact with the infant is restricted, or when the appearance of the infant provokes fear, as in this Spanish study:

*"Despite my psychological efforts I was not able to find that necessary connection to produce more and more milk"* (participant 10, Fernandez Medina et al 2019)<sup>90</sup>

Others prefer to distract themselves from the process using visual or auditory senses, for example by reading, listening to music or watching something on a device<sup>10,14</sup>.

A related concept is the pressure felt by mothers to produce milk and the distress and anxiety caused when volumes are limited<sup>11,29,74</sup>, demonstrated in these US and Scottish studies:

*"Here was this liquid gold I could be giving my infant, you know, fighting for their life, and I can't do it."* (anonymous participant, Palmquist et al 2020)<sup>29</sup>

*"You've obviously the worry of, 'Can I keep up with her?' You know every time you phone they say, 'We're putting her [milk] up another mil [milliliter],' and I'm thinking, 'Oh fantastic, oh no!'"* (anonymous participant, Swanson et al 2012)<sup>74</sup>

Inability to meet the infant's milk requirement is common, as discussed above. Mothers may feel objectified as simply a vehicle for milk production<sup>13,29</sup>, as seen in these studies from Spain, the USA and Finland:

*"I was just an exclusive provider of milk"* (participant 10, Fernandez Medina et al, 2019)<sup>90</sup>

*"I'm not just here to make milk, I want to participate in other ways of caring for my infant now."* (anonymous participant, Palmquist et al 2020)<sup>29</sup>

*"...it wasn't nice to hear daily the report of whether my own pumped milk was enough or not"* (participant 14, Niela-Vilen et al 2015)<sup>28</sup>

Acute anxiety is frequently reported by mothers to decrease their milk supply<sup>11,91,92</sup>, perhaps because of difficulty with continuing frequent expressions and reduced physical contact with the infant, as well as any direct effect of anxiety on lactation<sup>26</sup>. Episodes of health deterioration and maternal acute anxiety can be frequent for extremely preterm infants. This was noted in two USA qualitative studies focusing on Hispanic and non-Hispanic Black mothers:

*"I think I run out of milk because of the stress"* (anonymous participant, Parker et al 2018)<sup>88</sup>

*"When he got sick, I was stressed out. My mind was on, a mile a minute. It was crazy for me. It was very stressful, and my milk started to come down."* (anonymous participant, LoVerde et al 2018)<sup>92</sup>

## **2.3 An evolutionary perspective**

Breastmilk feeding can be viewed as a transaction, with the relative costs to mother and infant subject to evolutionary pressure. It is in the infant's genetic interest to extract as much nutritional and non-nutritional benefit from breastmilk as possible. In contrast, from the maternal perspective, energy spent on lactation impairs their own fitness and reproductive potential for future infants. This leads to a situation of mother-infant signalling to attempt to settle in the optimal evolutionary balance. Signalling can take place through physical contact and interaction. For example, crying influences the mother to bring the infant to the breast or the mother may employ other behaviours such as shushing and movement as an alternative soothing intervention instead of feeding. Signalling can also take place through the milk and breastfeeding process itself. For example, milk hormones may influence infant behaviour, and infant suckling intensity influences milk volume<sup>80</sup>.

The situation of very preterm birth disrupts mother-infant signalling in all its aspects by reducing physical contact with the infant and disconnecting the production of milk from the intake of milk. This may be another facet of the challenge placed on mothers attempting to provide breastmilk through expressing after preterm birth.



## 2.4 Relaxation interventions

A Cochrane systematic review identified relaxation as a promising intervention for mothers expressing milk<sup>32</sup>, and the impact of stress on lactation has been noted as a critical unresolved question in lactation research<sup>60</sup>. Although relaxation as a term is well understood by the public, it is difficult to define precisely. One definition is “a state of consciousness characterized by feelings of peace, and release of anxiety and fear”<sup>93</sup>. A state of perceived relaxation is associated with a constellation of physiological changes such as reduced heart rate, respiratory rate and blood pressure, and increased peripheral temperature, known as the ‘relaxation response’<sup>93</sup>. These changes are mediated by the parasympathetic nervous system, orientating the body towards a resting state focusing on digestion, in contrast to a ‘fight or flight’ arousal state focusing on efficient response to challenge or threat. The acute stress response is mediated by the sympatho-adreno-medullary axis and is marked by catecholamine release such as adrenaline<sup>93</sup>. In addition, the slower acting hypothalamo-pituitary-adrenal axis also mediates response to stress, marked by the secretion of stress hormones such as cortisol. There are therefore potential biochemical markers of relaxation such as reduction in cortisol as well as physiological markers such as heart rate and participant-reported subjective experience<sup>94</sup>.

Relaxation interventions are difficult to study because of their heterogeneity. Categories of relaxation interventions include<sup>93</sup>, but are not limited to:

- Progressive muscle relaxation (focus on relaxing skeletal muscles in sequence, often from head to toe)
- Meditation and mindfulness (emptying the mind; maintaining a deep, internal focus)
- Guided imagery (inducing thoughts of positive sensory experiences)
- Guided visualisation (cognitively simulating desired actions or feelings)
- Breathing exercises (often deep, slow and/or regular breathing)

Specific categories of relaxation are often combined into multicomponent interventions – for example starting with breathing exercises, then progressive muscle relaxation, then moving to

targeted visualisation. In contrast, another relaxation intervention might be simply listening to instrumental music. The combination of physical (progressive muscle relaxation/breathing exercises) and mental (imagery and visualisation) components may be particularly effective for some indications<sup>95</sup>.

Interventions may be condition-specific due to the nature of the guided visualisation – for example, visualising oneself as pain-free and enjoying activities that are otherwise limited by pain. Although related to relaxation, guided imagery and visualisation are separate entities with clear causal links to domain-specific physiological changes. For example, visualising food leads to an increase in saliva<sup>96</sup>.

Further sources of variability include the length of the treatment, the number of treatments and time period of delivery, the level of individualisation and participant choice, and the level of supervision of the participant by a practitioner. For example, a systematic review of RCTs using relaxation instruction for rehabilitation after ischaemic heart disease showed positive effects only for those studies using at least nine hours of instruction; ‘abbreviated’ instruction showed no long-term effects<sup>97</sup>. Per protocol dose response effects are also often reported<sup>98,99</sup> but are more difficult to interpret as they can be confounded by other characteristics of the participants causing them to comply well with relaxation practice. Personal experience of previous relaxation techniques, cultural attitudes and expectations can also impact on how relaxation therapy may be perceived by participants<sup>100</sup>.

Interventions that are targeted at inducing relaxation have been shown to improve depression<sup>101</sup>, anxiety<sup>97</sup> (including parents anxious about their child’s serious illness<sup>102</sup>) and chronic pain<sup>103–105</sup>. On a biochemical level, relaxation interventions can reduce cortisol<sup>99,106,107</sup>. On a physiological level, they can modify vital signs towards the expected ‘relaxation response’ constellation<sup>108,109</sup>. Relaxation interventions can increase subjective report of relaxation<sup>110</sup>. In general, research in the area of relaxation sometimes suffers from low scientific rigour, making robust conclusions difficult<sup>100</sup>.

### 2.4.1 Relaxation interventions and lactation

Research on relaxation interventions for lactation outcomes has been increasing in recent years<sup>111</sup>. Given the increased mental health difficulties faced by parents after very preterm birth, relaxation interventions may have independent value for this purpose. Mothers may value relaxation in connection to lactation in a more fundamental way, as described by this Taiwanese mother:

*"If I relaxed, my infant should feel the joy from my breast milk"* (anonymous participant, Lee et al 2009)<sup>16</sup>

Relaxation therapy could theoretically influence lactation outcomes through three main pathways; hormonal, neuronal and behavioural/cognitive. Firstly, relaxation could affect the hormones controlling milk production and release (prolactin and oxytocin). It should be noted that oxytocin itself also has a much broader function relating to forming relationships, bonding and parenting behaviour and is therefore inherently seen as a hormone inducing a state of relaxation, depending on the environment<sup>112</sup>. Another facet of complexity is that physiological stress responses are blunted in the perinatal period<sup>84</sup>, which emphasises the importance of research in the lactating population rather than extrapolation from wider studies (for example, the impact of relaxation on mental health).

There is a complex web of associations between mental health parameters, stress hormones such as cortisol and both prolactin and oxytocin<sup>79,113,114</sup>. Plasma levels of prolactin and oxytocin have sometimes<sup>85,115</sup>, but not always<sup>116</sup>, been negatively associated with anxiety and depression in both lactating and non-lactating women. Salivary cortisol and alpha-amylase (which correlates with catecholamine levels and is another marker of stress) were negatively associated with prolactin levels in mothers of very preterm infants over six weeks of expressing milk in one small study (n = 18)<sup>117</sup>. Measures of anxiety such as the State-Trait Anxiety Index (STAI) were shown to negatively correlate with oxytocin at 8 weeks after birth in another small study (n = 47)<sup>85</sup>. This evidence suggests that stress and lactation hormones are connected but does not give insight into the causal direction of the association.

In some settings there is a suggestion that lactation hormones influence stress. For example, markers of relaxation such as decreased blood pressure, heart rate, ACTH and cortisol response appear to chronologically follow breastfeeding or mechanical expression<sup>114</sup>. In one randomised controlled study, administration of nasal oxytocin (in a dose much higher than that induced by suckling) improved lactating mothers' report of their mood<sup>118</sup>.

However, there is also evidence of stress influencing lactation hormones. Acute stress due to induced maternal pain has been shown to impair infant milk intake in a historic experimental study with one participant. The effect was eliminated by administration of intramuscular synthetic oxytocin suggesting this may be the causal mechanism<sup>119</sup>. A more recent study confirmed that other (more ethical) forms of stress such as mental calculation and noise are associated with reduced suckling-related pulsatile oxytocin release<sup>83</sup>. Music therapy increased serum<sup>120</sup> and salivary<sup>121</sup> oxytocin in non-lactating people in line with subjective relaxation level and physiological signs of a relaxation response in two small randomised studies. This sparse evidence therefore suggests the possibility of relaxation interventions increasing oxytocin secretion, which then increases milk ejection. However, whether this could lead to a sustained increase in milk yield, rather than a transient response for one expressing or breastfeeding session, is not clear.

The second potential causal pathway is neurological. Mental visualisation of milk ejection can stimulate a central pathway to trigger the milk ejection reflex (MER, the muscular ejection of milk from the nipple), separately to the usual peripheral neuronal pathway stimulated by suckling or expression. For example, case studies of mothers with high spinal cord injury describe triggering MER with visualisation routines in the absence of any intact sensory pathways from the breast<sup>122</sup>. In a pilot RCT, some participants reported spontaneously experiencing MER when exposed to a relaxation recording that included lactation-specific visualisation, when their infants were not present<sup>108</sup>.

Operant conditioning is a second potentially relevant neuronal pathway – this refers to the process where a neutral stimulus or “conditioned reinforcer” (in this case, relaxation) is frequently paired

with a physiological stimulus or “primary reinforcer” (breastfeeding or milk expression) that triggers a physiological response (MER). Over time, the conditioned reinforcer can strengthen the physiological response, with or without the presence of the primary reinforcer. This is known to occur frequently in lactation, where a mother might experience oxytocin release and MER when her infant cries<sup>117</sup>. In the context of prolonged expressing, an increase in oxytocin levels is seen before the pump is applied to the breast, which is thought to be a manifestation of operant conditioning<sup>117</sup>.

Mothers of preterm infants often report difficulties with MER in qualitative studies<sup>11</sup>, due to the reduced physical contact and connection with their infants. Inhibition of MER is particularly damaging as it leads to incomplete drainage of the breast and therefore secondary inhibitory feedback of milk production<sup>113</sup>. Animal studies also support this mechanism – for example, a non-randomised study in cattle showed that playing instrumental music before and during milking was associated with increased behaviours indicating MER and increased rate of milk extraction<sup>123</sup>. Therefore, the influence of the central nervous system and operant conditioning are plausible pathways for the effect of repeated use of any ritualised intervention, and also specifically of lactation-specific guided visualisation.

Thirdly, a perception of relaxation may influence the mother’s breastfeeding self-efficacy and behaviour towards the infant. This could manifest in ways that directly affect lactation (for example increased frequency of expressing milk) or indirectly affect lactation (for example increased time spent in skin-to-skin contact). Mothers who have less perception and manifestation of stress are more likely to interact positively with their infant<sup>111</sup>, have increased self-efficacy<sup>124,125</sup>, better sleep quality<sup>126</sup>, and therefore may be more able and motivated to express effectively.

The condition-specific elements of complex relaxation interventions (i.e. visualisation and imagery) could theoretically affect the mother’s cognitive experience of the distress/lactation interface. For the very preterm population, guided visualisation that focuses on connection with the infant (visualising skin-to-skin contact and suckling) could be a source of psychological repair, shifting the

mother's perception of expressing towards the positive connection aspect and away from the negative alienation aspect discussed above<sup>10</sup>. Components of a relaxation intervention that emphasise the value of breastmilk to the infant attempt to increase the mother's satisfaction and positivity about the process of expressing<sup>127</sup>, although there is also a risk of a perception of pressure. The internal focus of most relaxation interventions attempts to combat the overwhelming sensory experience of the NICU. Some relaxation interventions contain elements that encourage parents to practice a facet of mindfulness labelled "non-reacting" ("allowing thoughts and feelings to come and go, without getting carried away by them"<sup>128</sup>). Mothers of healthy infants who scored higher for the trait of 'non-reacting' were less likely to stop breastfeeding over the first eight months after birth<sup>128</sup>.

Finally, the impact of relaxation interventions can alternatively be viewed with the evolutionary, signalling focused lens described above, particularly for mothers experiencing disrupted signalling after preterm birth. Guided visualisations of skin-to-skin contact and infant presence at the breast attempt to mimic signalling from the infant, thus potentially manipulating maternal physiology to invest in increased lactation resources. Additionally, using relaxation to shift the mother into a resting state may reduce energy expenditure and allow increased resource allocation to lactation.

## **2.5 The importance of evidence-based recommendations for expressing**

In the UK, mothers whose infants are not able to directly breastfeed are advised by the UNICEF UK Baby Friendly Initiative to start expressing within 2 hours of birth and to express 8-10 times a day for the entire expression period, including at least once in the night<sup>24</sup>. International guidance from the World Health Organisation and Scandinavian expert groups recommend first expression within 2-6 hours of birth and expressing at least 7 times in 24 hours<sup>129,130</sup>. These demanding schedules compete with the mother's other priorities, including caring for her infant, other children and herself<sup>29</sup>. However, these precise recommendations are predominantly extrapolated from the lactation physiology of mothers of term infants<sup>24,131,132</sup>. The general principle that early first

expression of milk and frequent expressing are associated with long-term lactation success are relatively well supported<sup>133</sup>. However, the specific clinical recommendations for these parameters are only loosely evidence based and there is low adherence in practice<sup>32–34</sup>. The specific justification provided by UNICEF UK is:

*“In order to maximise a mother’s potential to provide milk for her sick or preterm infant, it is essential to try to mimic what would happen if she had her ‘term’ baby with her from birth... mothers of preterm babies should be encouraged to... express a minimum of eight times in 24 hours, including once at night time... There is not a great deal of empirical evidence to suggest frequency of expression. However, extensive clinical experience recommends that a mother expresses between eight to ten times in a 24 hour period, not leaving a gap of more than six hours between expressions...”*

It could be argued that this guidance does not sufficiently consider the potential harms of these recommendations. Qualitative literature frequently finds themes related to the burden of expressing:

*“...the hours passed and just thinking about using that breast pump again, I wanted to cry”* (participant 13, Fernandez Medina et al 2019)<sup>90</sup>

*“I really want to take a nap but I need to pump. I really need to eat but I need to pump.”* (unidentified participant, Sisk et al 2010)<sup>14</sup>

*“...having to move back and forth. Being in public. Trying to get to the hospital. I think mainly it's just timing.... But at night it's with sleep. I'm so tired. I don't stay on schedule for every 2-4 hours.”* (unidentified participant, LoVerde et al 2018)<sup>92</sup>

*“They wanted me to pump every 2½ to 3 hours. I didn’t have enough time to do all that.”* (unidentified participant, Sisk et al 2010)<sup>14</sup>

*“Maintaining equipment... took hours and I was constantly extremely tired.”* (unidentified participant, Ikonen et al 2016)<sup>11</sup>

*“it's incredibly boring and hard work to keep at it”* (participant 4, Morelius et al 2020)<sup>75</sup>

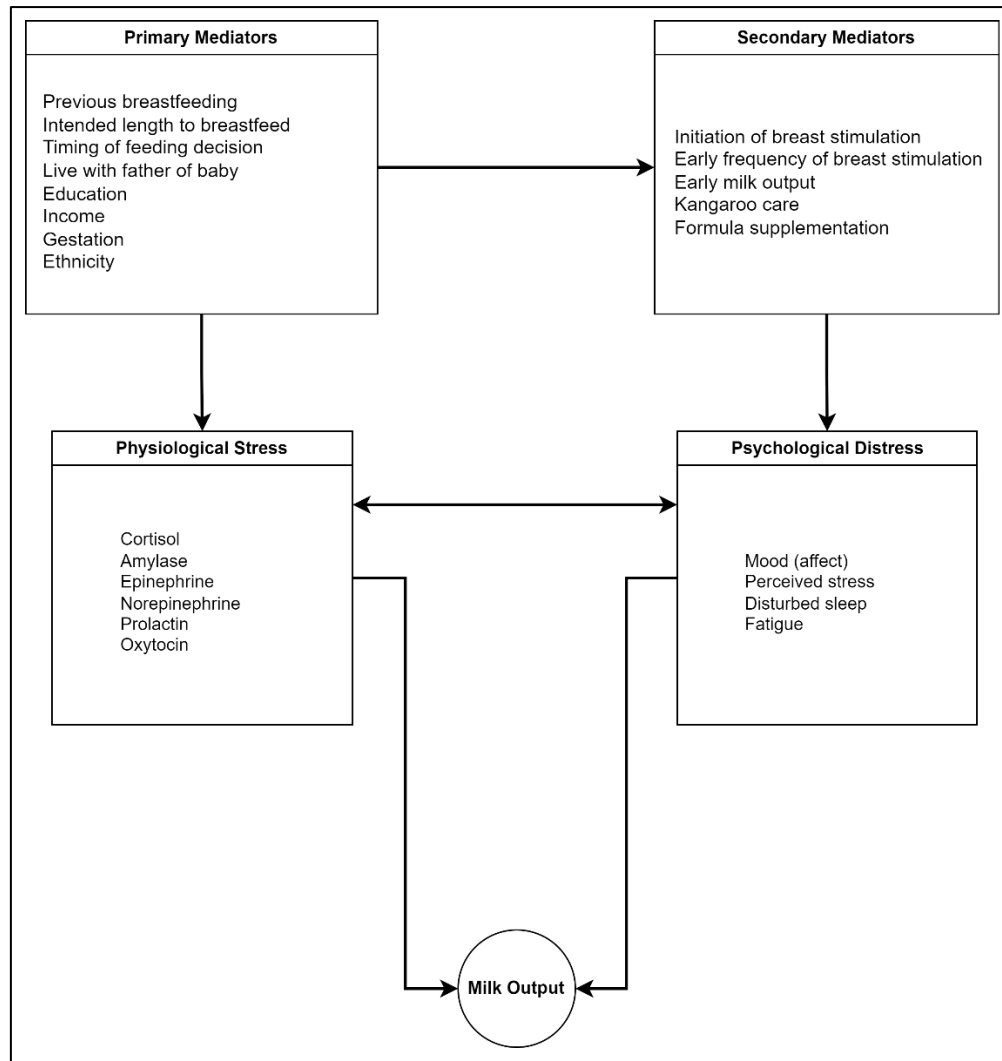
The burden of expressing can lead to cessation of lactation<sup>31</sup>. For example, in one study of 142 USA mothers of VLBW infants, 36% of those who had ceased lactation by six weeks after birth reported that this was due to time constraints and 13% said that it was related to competing motivations such as need for sleep<sup>30</sup>. Therefore, it is important to ensure that the specific recommendations are securely evidence-based and that parents can use quantitative evidence to weigh up the benefits for their long-term lactation potential against the immediate risks to physical and mental health.

## **2.6 Modifiable and non-modifiable influences on lactation**

Analysis of modifiable determinants of lactation such as expressing behaviour can only be performed with an understanding of the non-modifiable influences on lactation in this population. The Hill-Aldag model<sup>134</sup> (summarised in Figure 2.1) proposes that there are primary and secondary mediators (the terminology chosen by the authors) influencing long-term milk output. Proposed primary mediators included the mother's previous breastmilk feeding experiences, lactation plans, socioeconomic situation, support network, ethnicity and education, as well as the extent of prematurity. Although some of these factors are potentially modifiable at some point in the life course, they predominantly result from societal influences or are outside of the mother's immediate control. Proposed secondary mediators included time to initiation of breast stimulation, early frequency of breast stimulation, early milk output and extent of skin-to-skin contact with the infant. These factors remain largely within the locus of control of the mother and the healthcare team. The authors proposed that all of these variables act on long term milk quantity via physical and psychological stress.



**Figure 2.1: Hill-Aldag model<sup>134</sup> (summarised)**



Many studies support the impact of the following potential primary mediators on long-term milk output after very preterm birth (defined as any or exclusive breastmilk at or near discharge):

- Infant gestation<sup>38,58,65,135–148</sup>
- Infant birthweight<sup>38,58,65,91,141,142,144,145,147–151</sup>
- Maternal socioeconomic status<sup>30,137,148,150–154</sup>
- Maternal age<sup>31,37,65,136,137,141–144,149–156</sup>
- Maternal ethnicity<sup>31,39,137,141,142,152,153,155,157</sup>
- Maternal education<sup>30,31,39,137,148,154,158</sup>,
- Maternal smoking status<sup>30,135,136,138,143–145,148,149,151,154</sup>
- Presence of a partner<sup>136,137,148,152,154,159</sup>

- Maternal parity<sup>37,44,65,136,143,144</sup>
- Multiple birth<sup>65,138,142,145,147,160</sup>

The dose of skin-to-skin contact<sup>30,31,38,39,135,157,161</sup> with the infant is also an important variable with significant evidence for impact on milk output.

## 2.7 Summary

This chapter has described a population of very preterm infants at high risk of morbidity and mortality, and their mothers who are at high risk of mental health problems. Provision of mother's milk is important to reduce infant morbidity and mortality but is very challenging for mothers after very preterm birth and may impose increased distress in this setting. Successful provision of breastmilk additionally has the potential for psychological repair. The heterogeneous group of interventions classified as 'relaxation interventions' were described and potential mechanisms by which they could improve lactation outcomes were outlined. Finally, the burden of current clinical recommendations on milk expression and the context of modifiable and non-modifiable influences on lactation was presented. The next chapter will systematically review the evidence related to the effect of relaxation on lactation and wellbeing outcomes.

# **Chapter 3: The effect of relaxation interventions on lactation: a systematic review update with meta-analysis**

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## **3.1 Chapter Overview**

The field of relaxation interventions and lactation outcomes was systematically reviewed in 2016, without meta-analysis<sup>111</sup>. This chapter describes a systematic review update and subsequent meta-analysis, in order to achieve the first thesis objective. The aim of this systematic review update is to provide a comprehensive understanding of the evidence relating to the impact of relaxation interventions on lactation and mental health, to inform the design of the randomised study described in Chapter 5, and to provide the context to interpret its results. Of note, the most recent six studies<sup>162–167</sup> included here were published after the RCT protocol (Chapter 5) was finalised.

Much of the research presented in this chapter has been submitted for publication. I am the lead author of the submitted paper. The co-authors are the doctoral supervisors and the author of the prior systematic review. I adapted the original protocol, carried out the literature search, screened abstracts and full text reports, extracted data, performed risk of bias assessment and performed all the statistical analysis. The co-authors supported the protocol adaptation, and one carried out independent screening and risk of bias assessment.

### **3.1.1 Original systematic review**

The initial systematic review search took place in 2016, which is 6.5 years prior to the updated search. It showed limited evidence of the effectiveness of relaxation therapy on breastmilk composition and infant outcomes, with only five studies eligible for inclusion<sup>111</sup>. The field has evolved significantly since this time, providing further evidence to consider and allowing more sophisticated methods of synthesis. Although the impact of music therapy and lactation has been reviewed separately in 2020<sup>168</sup>, considering the wider field of all relaxation interventions allows a

more complete and nuanced exploration of this area. Additionally, a significant number of studies have been published between 2021 and 2023.

## 3.2 Methods

The systematic review update was conducted according to a pre-specified protocol and registered with Prospero (CRD 42021252986) in May 2021. For validity, the eligibility criteria and outcomes followed those set for the original review. Notably, this meant that there was no restriction on the gestational age at birth of infants.

### 3.2.1 Review outcomes

The primary questions are summarised in Table 3.1 in PICO format.

**Table 3.1: PICO model for primary systematic review questions**

|                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|-----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Population</b>           | Lactating individuals                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Intervention</b>         | Relaxation therapy                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Comparison</b>           | Control treatment or standard care                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Outcomes - primary</b>   | <ul style="list-style-type: none"> <li>• Duration and exclusivity of breastmilk feeding</li> <li>• Breastmilk quantity produced (for example, by expression, test weighing or labelled marker technique)</li> <li>• Nutritive milk constituents (for example, protein/energy)</li> <li>• Non-nutritive milk constituents (for example, cortisol)</li> <li>• Lactation hormone concentration (for example, oxytocin/prolactin in blood/saliva)</li> <li>• Infant growth parameters (for example, weight, height, head circumference, body composition)</li> <li>• Infant behaviour (for example, time spent crying/sleeping)</li> </ul> |
| <b>Outcomes - secondary</b> | <ul style="list-style-type: none"> <li>• Maternal mental health (for example, anxiety, stress, depression, post-traumatic stress)</li> <li>• Perception of relaxation</li> <li>• Infant feeding parameters (for example, frequency and duration)</li> <li>• Milk expression parameters (for example, frequency and duration)</li> <li>• Physiological markers of maternal stress (for example, cortisol in blood/saliva)</li> </ul>                                                                                                                                                                                                    |

Because there is very large heterogeneity in the way that the outcomes are measured in the literature and in clinical practice, the review protocol did not pre-specify the way in which each outcome should be defined, or its timing. General principles for meta-analysis, when multiple outcome data were reported in a single study, were to prefer standardised data, outcomes adjusted for baseline, and later outcomes if reported at several timepoints.

### 3.2.2 Search Strategy

The literature search matched the original review, using the following databases: Embase, Medline, CINAHL Plus, Allied and Complementary Medicine Database, Web of Science and the Cochrane Library. The search strategy used free text keywords and Medical Subject Headings (MeSH terms); the latter were specific to the classification used by each database whereas the former were consistent across databases. Example search terms are shown in Table 3.2 and full line by line search strategies are provided in Appendix Table A.1. Topic experts were also consulted for any other studies known to them. The final search took place on 24<sup>th</sup> February 2023.

**Table 3.2: Search strategy**

|   | Search strategy        | Keywords                                                                                       | Example MeSH terms                                                                                                              |
|---|------------------------|------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|
| 1 | Terms combined with OR | Breastfeeding, “breast feeding”, breastfed lactation<br>“breast milk”<br>“human milk”          | Breast feeding<br>Lactation<br>Human milk<br>Breast milk                                                                        |
| 2 | Terms combined with OR | “relaxation therapy”<br>meditation<br>“guided imagery”<br>“music therapy”<br>“verbal protocol” | Relaxation therapy, Relaxation techniques<br>Meditation<br>Imagery, Guided imagery<br>Music therapy, Music<br>Muscle relaxation |
| 3 | 1 AND 2                |                                                                                                |                                                                                                                                 |

### 3.2.3 Eligibility Criteria

Articles were eligible for inclusion if they were reported in full text in a peer-reviewed publication with an interventional design (randomised or non-randomised). Observational studies were excluded; use of a historical control group was considered observational. Studies were required to have a comparison group acting as a control, either blinded or unblinded. The update search was limited to papers published in 2016 or later. The original search had included an exclusion criteria for studies published in non-English language, however this was removed for the update search.

Studies could include lactating individuals who had given birth at any gestation and were feeding any number of infants/children in any location, with any parity. The protocol included a restriction

on the age of the infant at recruitment (under six months of age) but this was removed when it was noted that one of the studies in the original review<sup>169</sup> recruited mothers after this time point.

Studies could include any intervention primarily designed to achieve relaxation through reduction in mind-stress or psychological state. Specifically this would include meditation, mindfulness, guided imagery, visualisation, music therapy, aromatherapy, breathing exercises, muscle relaxation, applied relaxation, behavioural relaxation training, autogenic training, biofeedback and self-hypnosis. Pre-defined exclusion criteria were interventions primarily designed to achieve relaxation through reduction in body-stress (for example contact techniques such as massage), or aimed at cognitive and behavioural processes (for example cognitive behavioural therapy).

### **3.2.4 Study Quality Assessment**

Risk of bias was assessed independently by myself and a second researcher, Nurul Husna Mohd Shukri (NHMS), who was the author of the original systematic review<sup>111</sup>. DPhil supervisor Mary Fewtrell (MF) held consensus-directed discussions where needed. The Cochrane Risk of Bias tool (RoB-2)<sup>170</sup> was used as a structured tool to assess study quality and was applied to both the update and the original studies (this had not been performed in the original publication). An overall risk of bias was allocated to each outcome based on the highest risk assessment for any included domain. Graphical representations of risk of bias assessments were modified from those created within the RoB-2 Excel tool<sup>171</sup>.

Due to the nature of the interventions, blinding participants is inherently difficult. Where blinding does take place it must use a form of deception, such that people in either one or both allocation arms do not know that others are receiving a different treatment<sup>162,163,172</sup>. This is not possible in a setting where participants meet each other, such as a NICU, and there could be difficulties with adherence where participants have not received information on the possibility of being asked to use a relaxation intervention during the consent process. Therefore some settings will have no choice but to take an unblinded approach.

In unblinded settings, participant-reported outcomes with an element of subjectivity such as mental health questionnaires and records of infant behaviour were classified as at least ‘some concerns’ for bias. This was because participant belief in the intervention or desire to please the researchers could potentially impact participants’ report, consciously or unconsciously. Expressed milk quantity could be assessed as low risk of bias if derived from a 24-hour record or a single session of a specified length, in order to reduce the potential for the participant to modify the way they expressed solely for the outcome measurement (for example, expressing for longer).

### **3.2.5 Data Extraction**

The Covidence platform<sup>173</sup> was used to de-duplicate results and structure the screening and data extraction process for included studies. NHMS and I independently screened all abstracts and full text articles against the eligibility criteria. I extracted data from full text reports to a standardised form. Trial registration entries and published protocols were actively sought for each included study to inform the risk of bias assessments, as well as grey literature such as dissertation theses. Where complete information or a format of data required for meta-analysis was not available, all authors were given an equal opportunity to provide this; authors of eight studies did so<sup>108,163, 167, 172,174–176</sup>.

The template data extraction form is provided in Appendix Table A.2. In summary, this included study design, setting and time period, conflict of interest, protocol and/or trial registration, inclusion/exclusion criteria, number of participants, baseline characteristics, details of intervention and outcomes.

### **3.2.6 Methods for synthesis**

Fixed-effects (inverse variance) technique was used for meta-analysis, producing forest plots. Categorical outcomes were re-expressed as relative risks. Continuous outcomes were re-expressed as mean differences if data were reported on the same scale or standardised mean differences (Cohen’s method) if not. Standardised mean differences of 0.2 to 0.5 are considered small and 0.5 to 0.8 are considered medium<sup>177</sup>.

Where multiple forms of relaxation were included in separate arms of a single study, these were pooled to compare to the control group. If certain arms added multimodal interventions to relaxation that would not have fulfilled inclusion criteria (for example massage or looking at pictures of the infant), these arms were not included.

As recommended by the Cochrane handbook, outcomes that reported the change in data between two timepoints were combined in meta-analysis with those reporting data at a single timepoint only in unstandardised (not in standardised) analysis<sup>178</sup>. Case by case decisions were made when all available data for an outcome could not be combined in a single analysis because of these limitations, with sensitivity analysis performed for alternative options.

Where needed, paired data from crossover studies were derived from p values or t statistics<sup>179</sup>, as recommended by Cochrane<sup>178</sup>. If not possible, the study was included in unpaired format, which is a conservative approach likely to underweight crossover data<sup>178,179</sup>.

Potential publication bias was assessed with use of funnel plots if more than four studies were included in a meta-analysis. Statistical tests were not used because of the small number of studies<sup>180</sup>. It was expected that there would be substantial clinical heterogeneity as discussed above. Therefore an  $I^2$  statistic of more than 50% was considered to represent substantial statistical heterogeneity. Sensitivity analysis was performed using random effects meta-analysis for primary outcomes. Sensitivity analyses by risk of bias or study type were used on a case by case basis. Stata 18.0 was used for analysis.

The Grading of Recommendations, Assessment, Development and Evaluation (GRADE)<sup>181</sup> guidelines were used to assess the overall quality of evidence, using five domains to downgrade a baseline assumption of high certainty to moderate, low or very low certainty in the effect estimate. GRADE domains are risk of bias, inconsistency, imprecision, indirectness and publication bias. Quality assessment and summary of findings tables were prepared according to Cochrane recommendations<sup>178</sup>.

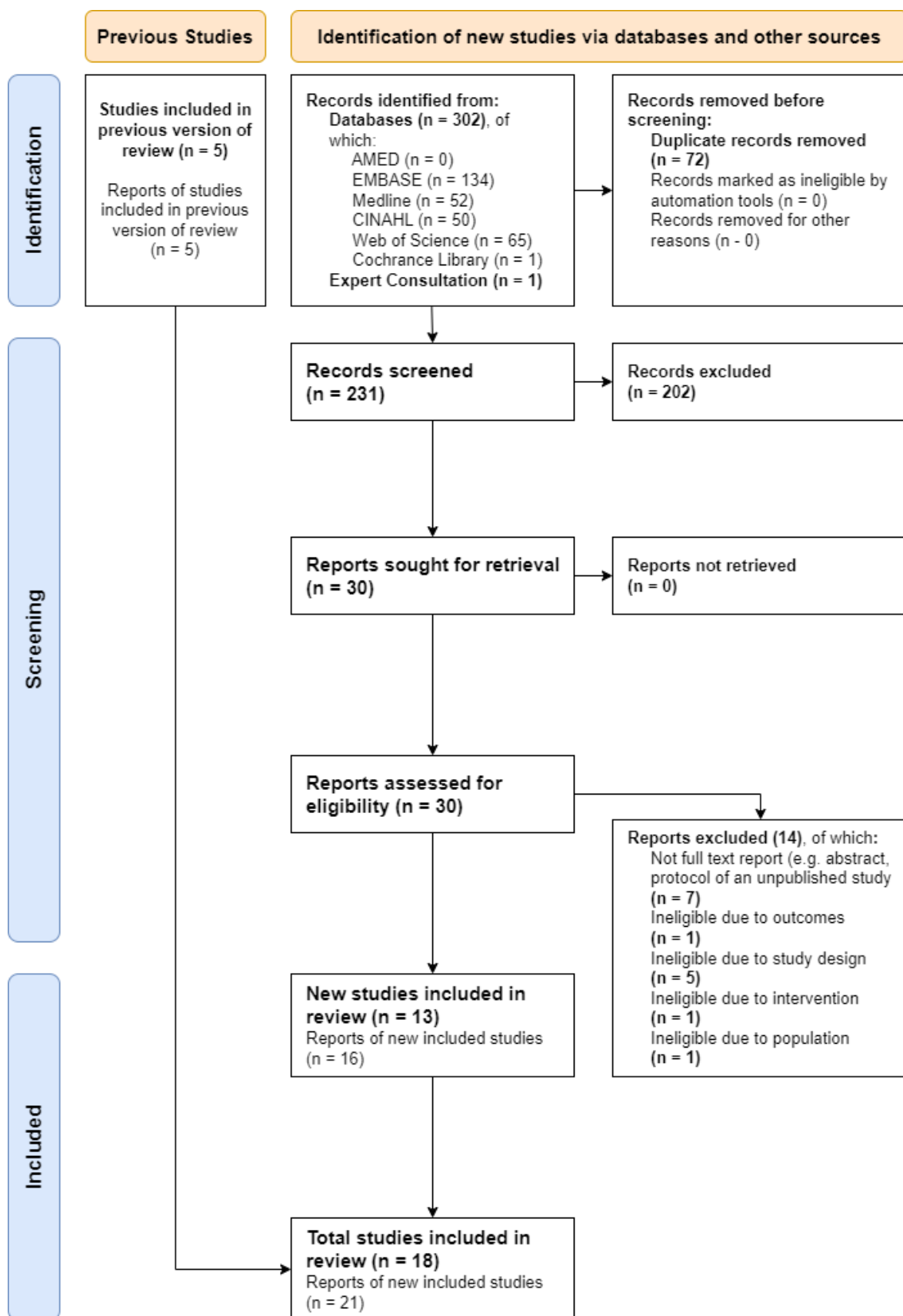


Pre-specified sub-groups for analysis and investigation of heterogeneity were defined for the review update. These were gestation at birth, mode of intervention delivery (self or provider), complexity of intervention (single or multicomponent), dose and timing.

### **3.3 Results**

The final search took place on 24<sup>th</sup> February 2023. Results are presented in PRISMA 2020<sup>182</sup> format in Figure 3.1. Database searches identified 302 records and one further record was added by a topic expert (published in a non-indexed journal<sup>176</sup>). After de-duplication, 231 individual records remained and 30 were assessed in full text review. Of these, fourteen reports were excluded due to ineligible format; abstract only<sup>183–189</sup>, ineligible outcome<sup>190</sup>, ineligible intervention<sup>191</sup> and ineligible population or study design<sup>126,192–194, 204</sup>. Also of note were three protocols that contributed additional information for included studies<sup>195–197</sup> and are included in the PRISMA chart as reports of included studies. The updated search therefore added sixteen reports of thirteen studies<sup>108,162–167,172,174,176,195–200</sup> to the five studies found in the original systematic review<sup>169,175,201–203</sup>.

**Figure 3.1: PRISMA 2020 flow diagram for systematic review update**



### 3.3.1 Included studies

Characteristics of included studies are summarised in Table 3.3. Appendix Table A.3 provides more detail on the design and outcomes of each study.

**Table 3.3: Summary of included studies**

| Study                                            | Design           | Sample size | Setting              | Infant gestation                     | Intervention (nature)                 | Intervention (dose)          | Original search? |
|--------------------------------------------------|------------------|-------------|----------------------|--------------------------------------|---------------------------------------|------------------------------|------------------|
| <b>Ak et al 2015</b> <sup>175</sup>              | Crossover RCT    | 30          | India 2012/13        | <34 weeks' (mean 32.4 <sup>1</sup> ) | Music (30 min)                        | Once a day for four days     | Yes              |
| <b>Chawanpaiboon et al 2021</b> <sup>198</sup>   | Parallel RCT     | 620         | Thailand 2018/19     | ≥37 weeks' (mean 38.5)               | Music (8 min)                         | Up to two days               | No               |
| <b>Dabas et al 2019</b> <sup>200</sup>           | Parallel RCT     | 57          | India 2017           | 26 to 33 weeks'                      | Relaxation practice (30 min)          | Once a day for ten days      | No               |
| <b>Dagli &amp; Celik 2022</b> <sup>165</sup>     | Crossover trial  | 73          | Turkey. Year unknown | <34 weeks'                           | Music (30 min)                        | Once only                    | No               |
| <b>Dib et al 2022</b> <sup>163</sup>             | Parallel RCT     | 72          | UK 2019-21           | 34 to 38 weeks' (mean 36.5)          | Relaxation recording (11 min)         | At least daily for two weeks | No               |
| <b>Feher et al 1989</b> <sup>202</sup>           | Parallel RCT     | 55          | USA. Year unknown    | <38 weeks' (mean 31.2)               | Relaxation recording (20 min)         | Daily for 8-11 days          | Yes              |
| <b>Keith et al 2012</b> <sup>203</sup>           | Parallel RCT     | 162         | USA. Year unknown    | <38 weeks'* (mean 31.9)              | Relaxation recording (12 min)         | 14 days                      | Yes              |
| <b>Kittithanesuan et al 2017</b> <sup>199</sup>  | Parallel RCT     | 304         | Thailand 2013        | ≥37 weeks' (mean 38.5)               | Music (11 min)                        | Once only                    | No               |
| <b>Massa et al 2022</b> <sup>164</sup>           | Parallel RCT     | 70          | USA 2018/19          | 24 to 32 weeks' (mean 30)            | Mindfulness meditation app (20 min)   | Daily, for nine days         | No               |
| <b>Mohd Shukri et al 2019</b> <sup>172,196</sup> | Parallel RCT     | 64          | Malaysia 2014        | ≥37 weeks'                           | Relaxation recording (15 min)         | At least daily for 12 weeks  | No               |
| <b>O'Connor et al 1998</b> <sup>201</sup>        | Controlled trial | 38          | USA. Year unknown    | "full term"                          | Relaxation recording (length unknown) | Two weeks                    | Yes              |
| <b>Perez-Blasco et al 2013</b> <sup>169</sup>    | Parallel RCT     | 26          | Spain 2012           | None specified                       | Mindfulness training (two hours)      | Weekly, for eight weeks      | Yes              |
| <b>Ramesh et al 2020</b> <sup>176</sup>          | Parallel RCT     | 81          | India. Year unknown  | ≥37 weeks'                           | Music (15 min)                        | Twice a day for 45 days      | No               |
| <b>SefidHaji et al 2022</b> <sup>167</sup>       | Parallel RCT     | 100         | Iran 2020            | 34 to 36 weeks' (mean 34.8)          | Music (30 min)                        | Once daily for six days      | No               |

<sup>1</sup> Provided by authors on request

| Study                                      | Design        | Sample size | Setting        | Infant gestation                 | Intervention (nature)               | Intervention (dose)              | Original search? |
|--------------------------------------------|---------------|-------------|----------------|----------------------------------|-------------------------------------|----------------------------------|------------------|
| <b>Shabnam et al 2021</b> <sup>166</sup>   | Parallel RCT  | 70          | Iran 2016/17   | BW 2 to 2.5kg (mean 36.8 weeks') | Music (5 min)                       | Three times a day for four weeks | No               |
| <b>Varisoglu et al 2020</b> <sup>174</sup> | Parallel RCT  | 44          | Turkey 2017/18 | 28 to 34 weeks' (mean 32)        | Music (15 min)                      | Twice a day for three days       | No               |
| <b>Yu et al 2023</b> <sup>162</sup>        | Parallel RCT  | 96          | China 2019/20  | 34 to 37 weeks' (mean 36.1)      | Relaxation recording (20 min)       | At least daily for 7 weeks       | No               |
| <b>Yu et al 2019</b> <sup>197</sup>        | Crossover RCT | 20          | China 2018     | None specified                   | a) Relaxation recording<br>b) Music | Once only                        | No               |

RCT = randomised, controlled trial. BW = birthweight. Min = minutes. \*or critically ill

The eighteen studies recruited 1982 participants, which is six times more than the original systematic review (311 participants). Fourteen randomised studies<sup>162–164,166,167,169,172,174,176,198–200,202,203</sup> were parallel group and two used a crossover design<sup>108,175</sup>. Two studies<sup>165,201</sup> were non-randomised interventional studies, one with a crossover design<sup>165</sup>.

Six studies<sup>108,162,163,172,202,203</sup> including 469 participants, used the same or very similar intervention recordings, guiding participants through muscle relaxation, breathing and visualisation/imagery specific to lactation. Two studies<sup>198,199</sup> including 924 participants, used one specific song with lyrics connected to breastfeeding and mothering (a Thai language song called Im-Oon). Six studies<sup>165–167,174–176</sup> including 398 participants, used instrumental music or lullabies. The remaining studies used a combination of yoga breathing exercises and muscle relaxation<sup>200</sup>, a mindfulness app<sup>164</sup>, mindfulness training<sup>169</sup> and relaxation training<sup>201</sup>.

Seven studies<sup>108,169,172,176,198,199,201</sup> included 1153 participants with full term or normal birthweight infants. Eleven studies<sup>162–167,174,175,200,202,203</sup> included 829 participants with preterm or low birthweight infants (two of these also included early term infants<sup>162,163</sup> and one also included critically ill term infants<sup>203</sup>).

Three trials declared in-kind support from a commercial company; a breast pump and bottle manufacturer<sup>108,172</sup> and the provider of a mindfulness app<sup>164</sup>.

### **3.3.2 Risk of Bias**

Figure 3.2 shows the risk of bias assessments for each outcome included in the systematic review.

Domains 1 and 2 (D1 and D2) apply to the entire study. An additional “domain S” – DS applies to the entire study for crossover studies only. DS assesses risk of bias from period and carryover; D1 the randomisation process and D2 deviations from the intended intervention due to the trial context. The remaining three domains (D3, D4 and D5) can differ for each outcome. D3 assesses missing outcome data; D4 measurement of the outcome and D5 selection of the reported result.

**Figure 3.2: Risk of bias score (Cochrane RoB-2) for overall outcome and each domain**

| Study                     | Outcome                                                | DS | D1 | D2 | D3 | D4 | D5 | Overall |
|---------------------------|--------------------------------------------------------|----|----|----|----|----|----|---------|
| Ak et al 2015             | Expressed milk volume                                  | ⊖  | !  | +  | +  | +  | !  | ⊖       |
|                           | Salivary cortisol                                      | ⊖  | !  | +  | +  | ⊖  | !  | ⊖       |
| Chawanpaiboon et al 2022  | Exclusive breastfeeding                                |    | ⊖  | ⊖  | +  | +  | ⊖  | ⊖       |
| Dabas et al 2019          | Mental health scores & expressed milk volumes          |    | +  | !  | !  | !  | +  | !       |
| Dagli & Celik 2022        | Expressed milk volume                                  | ⊖  | ⊖  | +  | +  | +  | !  | ⊖       |
|                           | Mental health scores                                   | ⊖  | ⊖  | +  | +  | !  | !  | ⊖       |
| Dib et al 2022            | Stress score & infant weight gain                      |    | +  | +  | +  | +  | +  | +       |
|                           | Depression score & infant length gain                  |    | +  | +  | !  | +  | +  | !       |
|                           | Milk intake, salivary cortisol, infant behaviour       |    | +  | +  | ⊖  | !  | +  | ⊖       |
|                           | Milk macronutrients                                    |    | +  | +  | !  | +  | +  | !       |
| Feher et al 1989          | Milk fat                                               |    | ⊖  | +  | ⊖  | +  | !  | ⊖       |
|                           | Expressed milk volume                                  |    | ⊖  | +  | ⊖  | !  | !  | ⊖       |
| Keith et al 2012          | Expressed milk volume & fat                            |    | !  | +  | +  | +  | +  | !       |
| Kittithanesuan et al 2017 | Colostrum score                                        |    | +  | +  | +  | !  | !  | !       |
| Massa et al 2022          | Mental health scores                                   |    | +  | +  | !  | !  | +  | !       |
|                           | Any breastfeeding, milk intake, expressing behaviour   |    | +  | +  | !  | +  | +  | !       |
| Mohd Shukri et al 2019    | Mental health scores & infant growth                   |    | +  | +  | +  | +  | +  | +       |
|                           | Milk intake & cortisol                                 |    | +  | +  | +  | +  | !  | !       |
|                           | Infant behaviour & body composition                    |    | +  | +  | ⊖  | +  | +  | ⊖       |
| O'Connor et al 1998       | Milk IgA                                               |    | ⊖  | +  | +  | +  | ⊖  | ⊖       |
| Perez-Blasco et al 2013   | Mental health scores                                   |    | !  | +  | +  | !  | !  | !       |
| Ramesh et al 2020         | Time to lactogenesis II                                |    | !  | +  | !  | !  | ⊖  | ⊖       |
| SefidHaji et al 2022      | Expressed milk volume & constituents                   |    | +  | +  | +  | +  | +  | +       |
| Shabnam et al 2021        | Feeding and output diary                               |    | ⊖  | +  | ⊖  | ⊖  | !  | ⊖       |
|                           | Infant behaviour                                       |    | ⊖  | +  | ⊖  | ⊖  | ⊖  | ⊖       |
|                           | Infant growth                                          |    | ⊖  | +  | ⊖  | +  | ⊖  | ⊖       |
| Varisoglu et al 2020      | Expressed milk volume, mental health & cortisol        |    | !  | !  | !  | +  | ⊖  | ⊖       |
| Yu et al 2019             | Vital signs                                            | +  | +  | +  | +  | +  | +  | +       |
|                           | Report of relaxation                                   | +  | +  | +  | +  | !  | +  | !       |
| Yu et al 2023             | Mental health scores, milk constituents, infant growth |    | +  | +  | +  | +  | +  | ⊖       |
|                           | Milk intake & infant behaviour                         |    | +  | +  | ⊖  | !  | +  | +       |

**Key:** Low risk (+) Some concerns (!) High risk (⊖)

DS = Domain 5 (period and carryover, crossover studies only); D1 = Domain 1 (randomisation); D2 = Domain 2 (deviations from intended interventions); D3 = Domain 3 (missing outcome data); D4 = Domain 4 (outcome measurement); D5 = Domain 5 (result selection)

### 3.3.2.1 Summary of risk of bias

Eleven out of eighteen studies were classified as high risk of bias for at least one outcome<sup>162,163,165,166,172,174–176,198,201,202</sup>. Five studies reported at least one outcome classified as low risk of bias<sup>108,162,163,167,172</sup>.

Two (of three) crossover studies<sup>165,175</sup> were high risk of bias in Domain 5 due to inadequate washout period. Five studies were high risk of bias in domain one because of concerns over randomisation<sup>198</sup>, inadequate allocation concealment<sup>174,202</sup> or non-randomised design<sup>165,201</sup>. One study was high risk of bias in domain two due to exclusion of participants lost to follow up from all parts of the analysis<sup>198</sup>.

Five studies<sup>162,163,166,172,202</sup> had at least one outcome classified as high risk of bias for domain three due to the level of missing data and/or the balance of missing data between allocation arms. Two studies<sup>166,175</sup> had at least one outcome classified as high risk of bias for domain four due to lack of definition of how the outcome was assessed. Five studies had at least one outcome classified as high risk of bias for domain five due to concerns over selective classification or reporting of results<sup>166,174,176,198,201</sup>.

### **3.3.3 Results Summary**

Table 3.4 provides an overall summary of the meta-analysis results for each outcome reported by at least one study, showing both the effect estimates and GRADE certainty of evidence. Complete data tables are provided for each outcome in Appendix Table A.4. Funnel plots for all meta-analyses with more than four studies included are provided in Appendix Figure A.1.

**Table 3.4: Summary of meta-analysis outcomes**

| Milk constituents                  |                                    |                                |                                     |                                     |
|------------------------------------|------------------------------------|--------------------------------|-------------------------------------|-------------------------------------|
| Protein (g/100 mL)                 | Carbohydrate (g/100 mL) ↑          | Energy (kcal/100 mL) ↑         | Fat (g/100 mL)                      | Cortisol (%) ↓                      |
| MD: 0.00 (0.00, 0.00)              | MD: <b>0.15</b> (0.01, 0.29)       | MD: <b>1.84</b> (0.10, 3.57)   | SMD: 0.17 (-0.04, 0.37)             | MD: <b>-44.5</b> (-76.1 to -12.9)   |
| Feeding outcomes                   |                                    |                                |                                     |                                     |
| Milk quantity ↑                    | Exclusive breastmilk               | Any breastmilk                 | Expressing frequency                | Time to secretory activation ↓      |
| SMD: <b>0.73</b> (0.57, 0.89)      | RR: 0.99 (0.90, 1.09)              | RR: 1.18 (0.89, 1.58)          | MD: 1 per day (-1 to 1.5)           | MD: <b>-1.1 days</b> (-1.7 to -0.6) |
| Infant growth                      |                                    |                                |                                     |                                     |
| Weight (SDS change) ↑              | Length (SDS change)                | Head circumference             | Fat-free mass (kg)                  | Fat mass (kg)                       |
| MD: <b>0.51</b> (0.30 to 0.72)     | MD: 0.04 (-0.21 to 0.29)           | MD: 0.27 cm (-0.53 to 1.07)    | MD: 0.5 (-0.06 to 1.06)             | MD: 0.4 (-0.05 to 0.75)             |
| Infant Behaviour                   |                                    |                                | Maternal physiology                 |                                     |
| Sleeping (mins/day) ↑              | Crying (mins/day)                  | Awake/alert (mins/day) ↑       | Vital signs e.g. diastolic BP ↓     | Salivary cortisol (nmol/L)          |
| MD: <b>53.6</b> (41.8 to 65.3)     | MD: -5.2 (-30.8 to 20.4)           | MD: <b>38.3</b> (18.2 to 58.4) | MD: <b>-5.9 mmHg</b> (-9.1 to -2.8) | MD: -0.34 (-0.73 to 0.05)           |
| Maternal wellbeing                 |                                    |                                |                                     |                                     |
| Stress score ↓                     | Anxiety score ↓                    | Depression score               | Relaxation (VAS) ↑                  | Breastfeeding self-efficacy*        |
| SMD: <b>-0.49</b> (-0.70 to -0.27) | SMD: <b>-0.47</b> (-0.66 to -0.29) | SMD: -0.23 (-0.56 to 0.10)     | MD: <b>1.1</b> (0.43 to 1.7)        | Med Diff: 0 (-7.5 to 5)             |
| Key:                               |                                    |                                |                                     |                                     |
|                                    | High certainty                     | Moderate certainty             | Low certainty                       | Very low certainty                  |

Significant results are bolded ( $p < 0.05$ ), arrow shows direction of effect. MD = mean difference. SMD = standardised mean difference. RR = relative risk. SDS = standard deviation score. VAS = Visual Assessment Scale. BP = blood pressure. \*Measured with the Breastfeeding Self-efficacy Scale (short form for NICU)

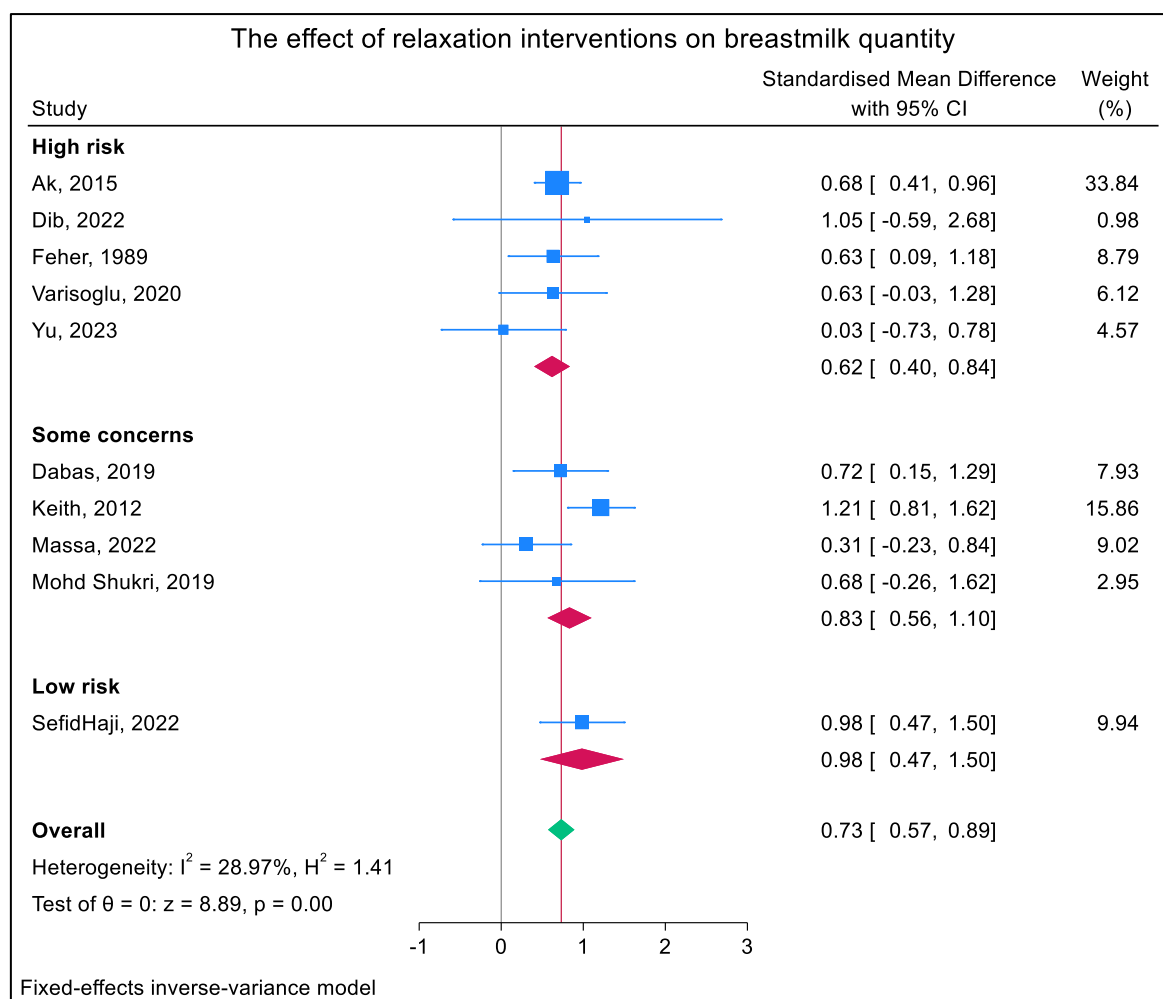
### 3.3.4 Primary Outcomes

#### 3.3.4.1 Breastmilk quantity

There was **moderate certainty** evidence that relaxation led to an increase in breastmilk quantity (standardised mean difference; SMD 0.73, 95% CI 0.57 to 0.89,  $p < 0.0001$ ; 10 studies, 465 participants; Figure 3.3). This is an increase in milk quantity of 0.73 standard deviations, which is considered a medium effect size<sup>177</sup>. The level of statistical heterogeneity did not exceed the pre-defined threshold ( $I^2$  of 29%).



**Figure 3.3: Forest plot for breastmilk yield, grouped by risk of bias assessment**



The GRADE assessment for the pooled effect estimate was moderate certainty (downgraded by one level for risk of bias).

These ten RCTs measured milk quantity expressed in NICU settings<sup>164,167,174,175,200,202,203</sup> or drunk by a healthy infant at the breast (through the use of deuterium isotope or test weighing)<sup>162,163,172</sup>. The outcome measurement unit was either one expression session or a 24-hour period. One study (Mohd Shukri et al 2019)<sup>172</sup> provided both unadjusted and adjusted mean difference. Although the general pre-specified principle was to use adjusted data, in this case the unadjusted data was used because the adjustment was post-hoc (requested by manuscript reviewers). The adjusted increase in milk quantity was larger than the unadjusted effect estimate, and reached statistical significance, so this was the conservative choice.

The studies used lactation-specific guided relaxation<sup>162,163,172,202,203</sup>, instrumental music or lullabies<sup>167,174,175</sup>, yoga breathing exercises<sup>200</sup> and a mindfulness app<sup>164</sup>. Five studies were at high risk of bias for this outcome (due to insufficient washout period in a crossover study<sup>175</sup>, level of missing outcome data<sup>162,163,202</sup> and selection of reporting<sup>174</sup>). As can be seen in Figure 3.3, there was little difference in effect estimate by risk of bias; in fact the trend was towards studies at lower risk of bias having higher effect estimates.

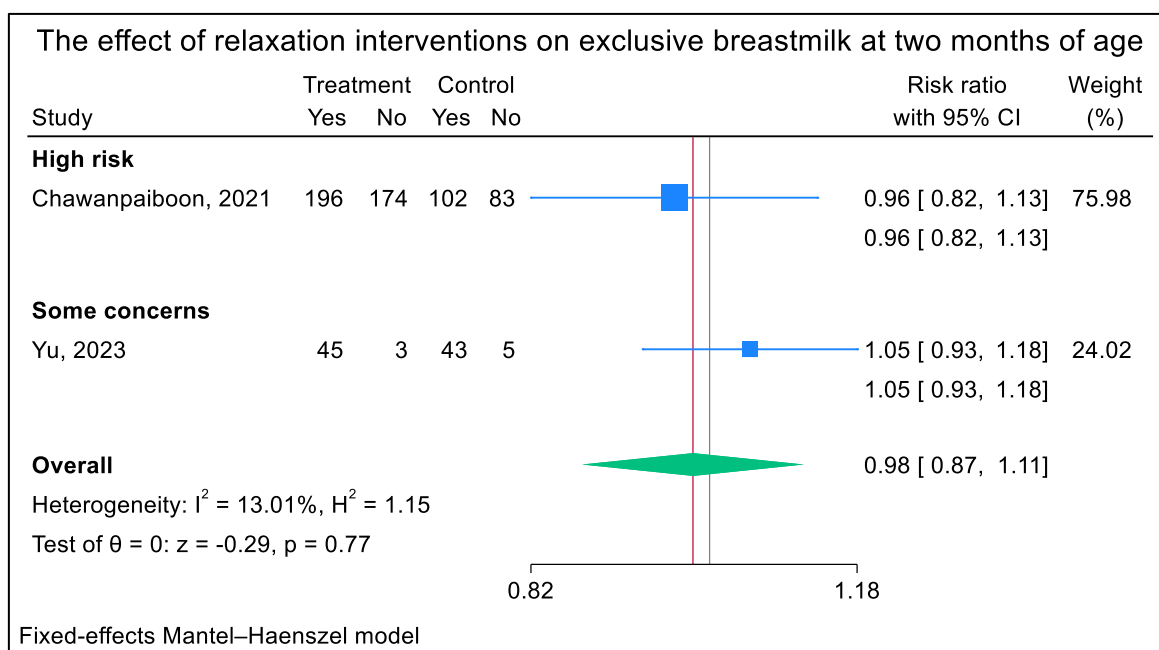
One further non-randomised crossover study<sup>165</sup> using music in a NICU setting was not included in meta-analysis due to concerns about bias from the expected increase of milk quantity over time. All participants received the intervention condition two days after the control condition, at a time when milk yield is expected to increase day by day.

#### 3.3.4.2 *Rate and exclusivity of breastfeeding*

There was **low certainty** evidence of no difference in the proportion receiving any breastmilk at one month of age (RR 1.18, 95% CI 0.89 to 1.58, 47 participants) in a single study. This study was classified as some risk of bias due to missing data. The intervention was a mindfulness app after very preterm birth<sup>164</sup>.

There was **low certainty** evidence of no difference in the proportion receiving exclusive breastmilk at two months of age (RR 0.98, 95% CI 0.87 to 1.11; 2 studies, 651 participants; Figure 3.4). The two studies contributing to this outcome provided music for up to 48 hours after birth<sup>198</sup> or lactation-specific guided relaxation for several weeks<sup>162</sup>. The former study was at high risk of bias due to concerns with the randomisation process<sup>198</sup>.

**Figure 3.4: Forest plot for exclusive breastmilk feeding at two months after birth, grouped by risk of bias assessment**



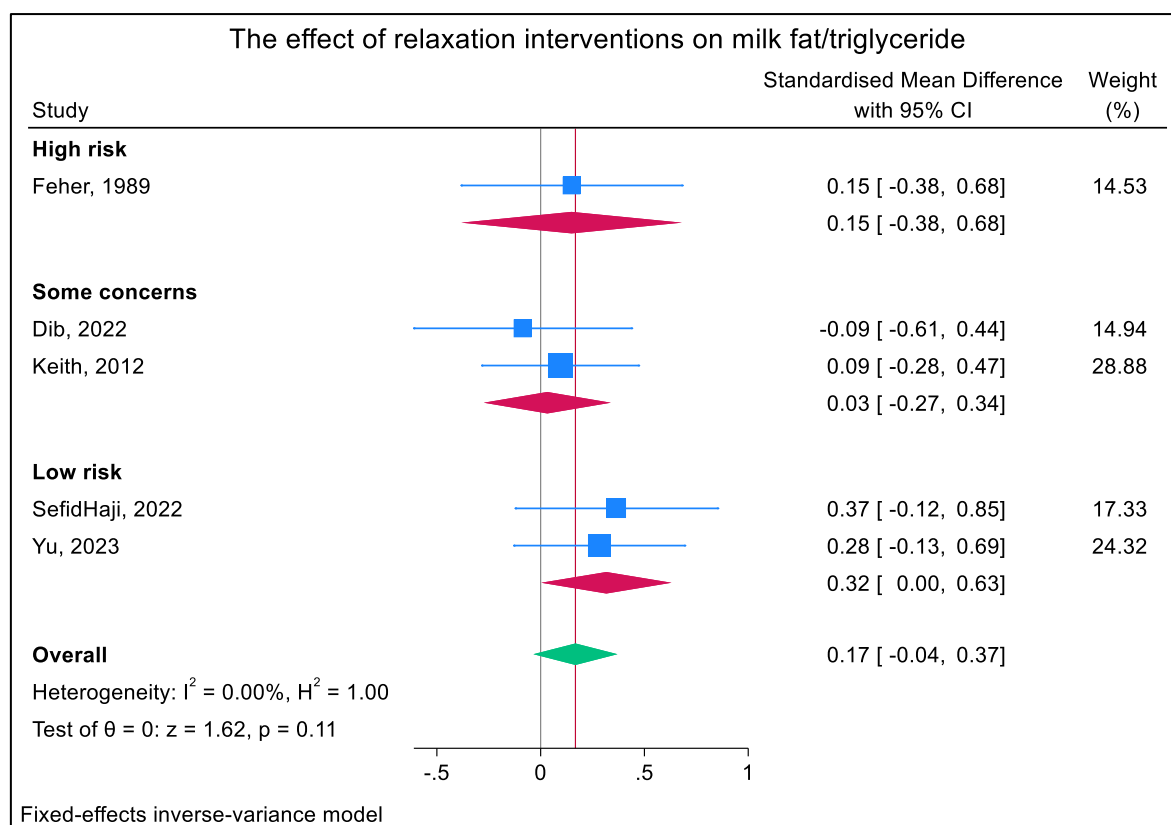
The GRADE assessment for the pooled effect estimate was low certainty (downgraded by one level for risk of bias and one level for imprecision).

### 3.3.4.3 Milk constituents

#### 3.3.4.3.1 Milk fat

There was **low certainty** evidence of no difference in milk fat (SMD 0.17, 95% CI -0.04 to 0.37; 5 studies, 388 participants; Figure 3.5). The five RCTs reported data on milk fat<sup>162,163</sup>, creatatocrit<sup>202,203</sup> or triglyceride<sup>167</sup> after between six days and eight weeks of music<sup>167</sup> or lactation-specific guided relaxation<sup>162,163,202,203</sup>. Composite (mixed)<sup>162,163,167,202,203</sup> or foremilk samples<sup>162</sup> were taken between 10am and noon<sup>162,163,167,202,203</sup> or at an unspecified time<sup>202</sup>. Three studies took place in the NICU setting<sup>167,202,203</sup> and two in the community, after late preterm and early term birth<sup>162,163</sup>. One study was at high risk of bias due to concern over allocation concealment and unequal missing data<sup>202</sup>. Two others had some concern over risk of bias<sup>163,203</sup>.

**Figure 3.5: Forest plot for breastmilk fat, grouped by risk of bias assessment**



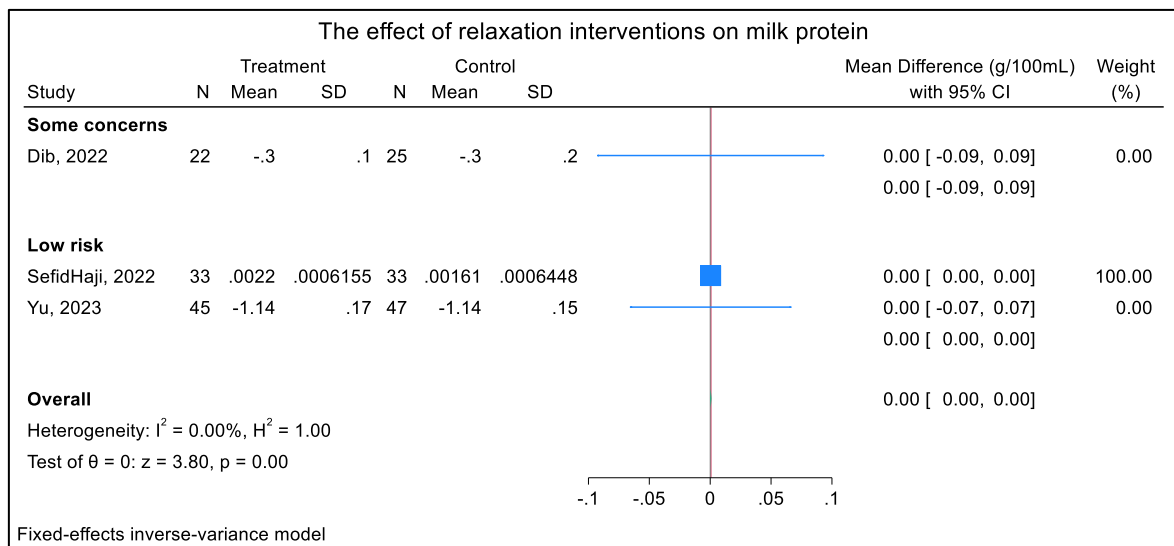
The GRADE assessment for the pooled effect estimate was low certainty (downgraded by one level for risk of bias and one level for imprecision).

### 3.3.4.3.2 Milk protein

There was **high certainty** evidence of no difference in milk protein (MD 0.00 g/100mL, 95% CI 0.00 to 0.00; 3 studies, 205 participants; Figure 3.6). The three RCTs reported change in milk protein (Figure 3.6) over a variety of time periods from six days to eight weeks<sup>162,163,167</sup>. One study was classified as some concerns for risk of bias due to missing data<sup>163</sup>.

Although one study<sup>167</sup> dominates the meta-analysis due to a very low standard deviation, removing this study produces the same conclusion (MD 0.00 g/100mL (95% CI -0.05 to 0.05, 2 studies, 139 participants). Notably, the milk protein concentration in the dominant study was much lower than expected. The authors confirmed in personal communication that there was no error in the units reported but offered no explanation for why the protein concentration was lower than published ranges for human milk.

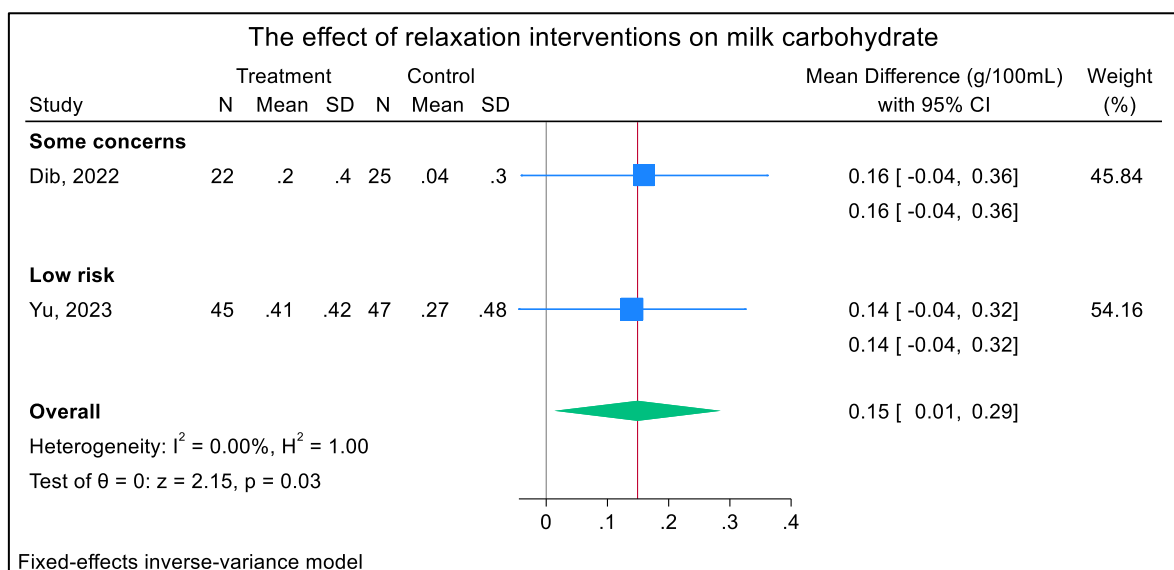
**Figure 3.6: Forest plot for milk protein (change between two timepoints, in g/100 mL), grouped by risk of bias assessment**



### 3.3.4.3.3 Milk carbohydrate and energy

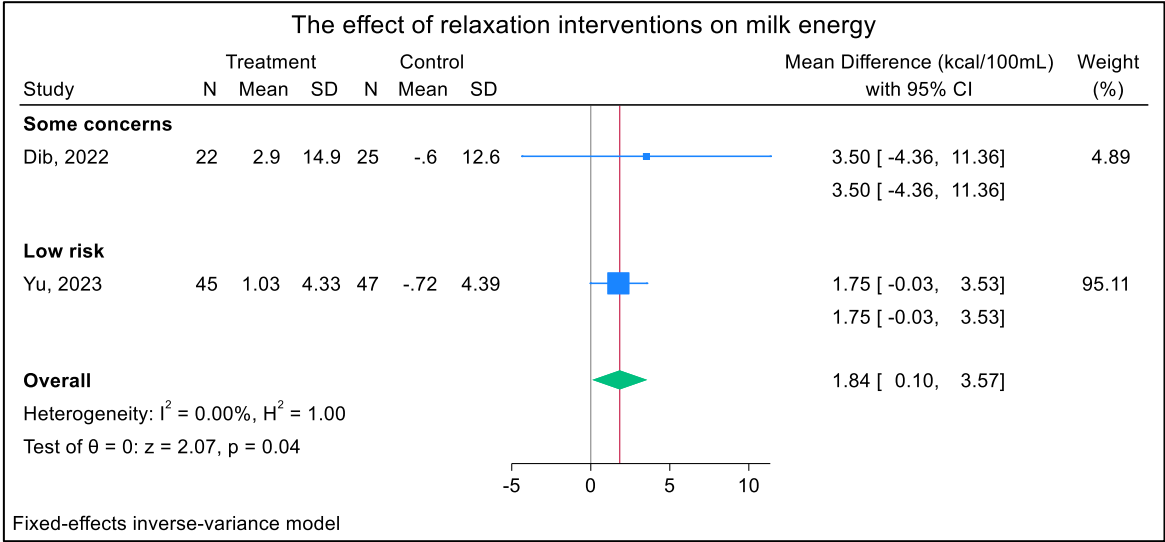
There was **moderate certainty** evidence of a small increase in milk carbohydrate (MD 0.15 g/100mL, 95% CI 0.01 to 0.29,  $p < 0.0001$ ; 2 studies, 139 participants; Figure 3.7) and milk energy (MD 1.84 kcal/100mL, 95% CI 0.10 to 3.57,  $p < 0.0001$ ; 2 studies, 139 participants; Figure 3.8). Two RCTs contributed to these two outcomes, reporting change in milk carbohydrate and energy from a baseline of week 1-3 after birth to a post-intervention week 6-8. One study was classified as some concerns for risk of bias due to missing data<sup>163</sup>.

**Figure 3.7: Forest plot for milk carbohydrate (change between week 1-2 and week 6-8, in g/100 mL), grouped by risk of bias assessment**



The GRADE assessment for the pooled effect estimate was moderate certainty (downgraded by one level for risk of bias).

**Figure 3.8: Forest plot for milk energy (change between week 1-2 and week 6-8, in kcal/100 mL), grouped by risk of bias assessment**



The GRADE assessment for the pooled effect estimate was moderate certainty (downgraded by one level for risk of bias).

### 3.3.4.3.4 Milk cortisol

There was **low certainty** evidence of a reduction in hindmilk cortisol after the first exposure to lactation-specific guided relaxation (-44.5%, 95% CI -76.1 to -12.9; 1 study, 63 participants), but not after six weeks of regular use, in a single study. This study was classified as some concern for risk of bias due to a change in the format of the outcome compared to the protocol<sup>172</sup>.

The GRADE assessment for the effect estimate was low certainty (downgraded by one level for risk of bias and one level for imprecision).

### 3.3.4.3.5 Milk IgA

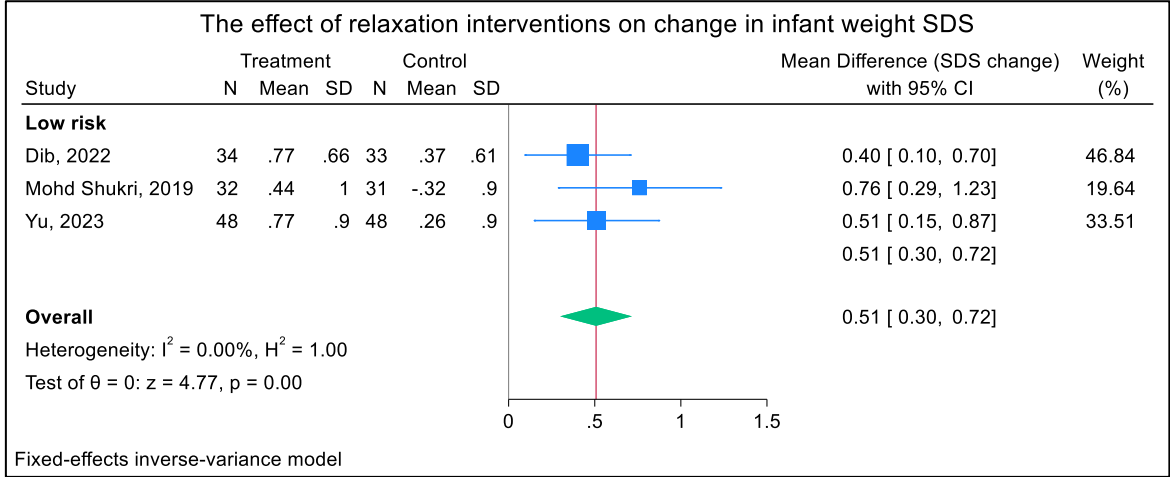
There was **very low certainty** evidence of no difference in milk IgA after relaxation training for mothers of term infants (MD-0.01 g/L, 95% CI -0.08 to 0.06, 1 study, 28 participants). This outcome is at high risk of bias due to non-randomised design<sup>201</sup>.

The GRADE assessment for the effect estimate was very low certainty (downgraded by two levels for risk of bias and one level for indirectness).

### 3.3.4.4 Infant Growth

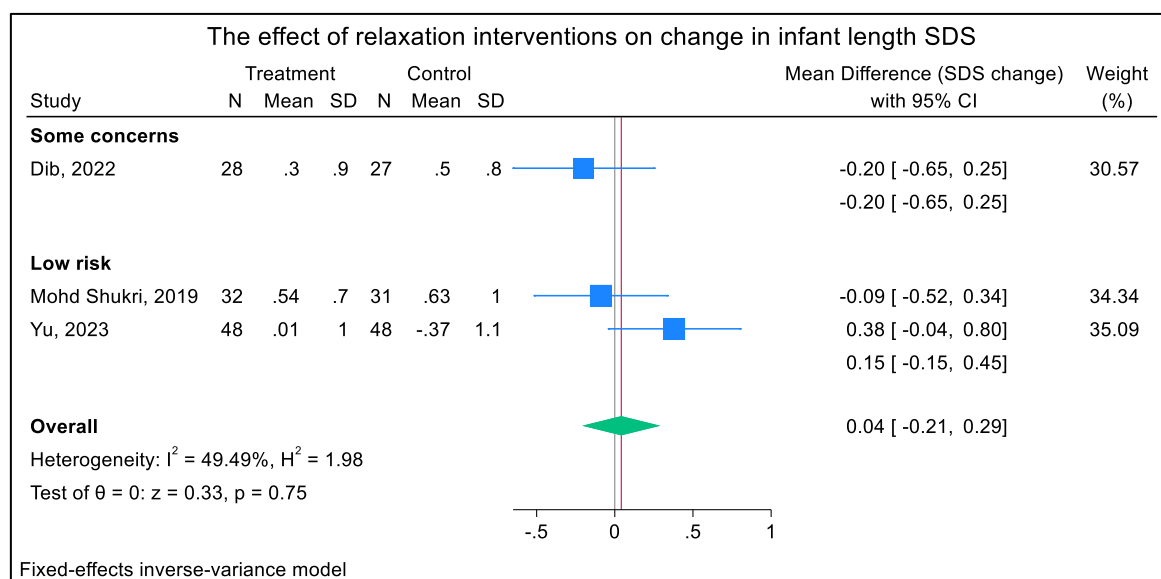
There was **high certainty** evidence of an increase in infant weight (MD 0.51 change in standardised difference score; SDS, 95% CI 0.30 to 0.72,  $p<0.0001$ ; 3 studies, 226 participants; Figure 3.9). For comparison, an SDS/z-score change of 0.67 is equivalent to moving between the 25<sup>th</sup> and 50<sup>th</sup> centiles on a population growth chart, so this is a moderate increase. All of the included studies were in the context of direct breastfeeding.

**Figure 3.9: Forest plot for the change in infant weight SDS between the ages of 1-2 weeks and 6-12 weeks, grouped by risk of bias assessment**



There was **moderate certainty** evidence of no change in infant length (MD of 0.04, 95% CI -0.21 to 0.29; 3 studies, 214 participants; Figure 3.10). There was some heterogeneity, although not quite exceeding the pre-specified level ( $I^2$  of 49.5%).

**Figure 3.10: Forest plot for the change in infant length SDS between the age of 1-2 weeks and 6-12 weeks, grouped by risk of bias assessment**



The GRADE assessment for the pooled effect estimate was moderate certainty (downgraded by one level for inconsistency).

Three RCTs contributed to these two outcomes, reporting change in standard deviation scores (SDS or z score) between 1-2 and 8-12 weeks after birth. These studies all used modifications of the same lactation-specific guided relaxation<sup>162,163,172</sup> after term or late preterm birth. One<sup>163</sup> was classified as some concerns for the outcome of length only, due to missing data.

A further RCT reported infant weight, length and head circumference at 28 days after birth in grams and centimetres respectively. This study was at high risk of bias due to an imbalance of missing data and concerns over selection of the outcomes reported<sup>166</sup>.

All four studies could instead be combined in standardised meta-analysis, which would increase the effect estimate (infant weight SMD 0.77, 95% CI 0.53 to 1.01,  $p < 0.001$ , 4 studies, 284 participants; infant length SMD 0.46, 95% CI 0.22 to 0.71,  $p < 0.001$ , 4 studies, 272 participants). However the heterogeneity is very high ( $I^2 = 71\%$  and  $88\%$  respectively), which questions the validity of combining raw and standardised outcome measures through meta-analytical standardisation, particularly in the context of the addition of a study at high risk of bias<sup>166</sup>. Therefore the initial meta-analysis above is considered more valid.



There was **very low certainty** evidence for no change in infant head circumference in the single study at high risk of bias<sup>166</sup> (MD 0.27cm, 95% CI -0.53 to 1.07, 1 study, 58 participants).

The GRADE assessment for the effect estimate was very low certainty (downgraded by one level for imprecision and two levels for risk of bias).

There was **very low certainty** evidence for no change in infant body composition in a single study at high risk of bias due to large and unbalanced loss to follow up<sup>172</sup>. This was the case for fat free mass (MD 0.5kg, 95% CI -0.06 to 1.06; 1 study, 29 participants) and for fat mass (MD 0.4kg, 95% CI -0.05 to 0.75; 1 study, 29 participants).

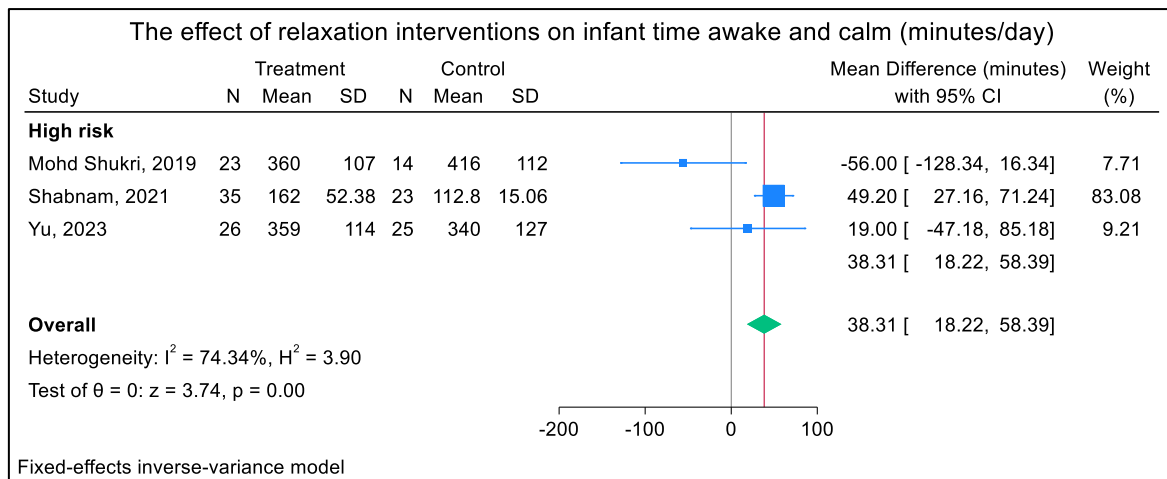
The GRADE assessment for the effect estimate was very low certainty (downgraded by two levels for risk of bias and one level for imprecision).

#### 3.3.4.5 *Infant Behaviour*

Four RCTs<sup>162,163,166,172</sup> reported infant behaviour at between four and eight weeks of age, although not all studies reported on all types of behaviour. Three<sup>162,163,166,172</sup>, all similar studies with different modifications of the same lactation-specific guided relaxation, used an established three day infant diary where parents classify infant behaviour in 15 minute epochs. The other study, which used instrumental music, did not state the format of recording and apparently recorded infant behaviour for 28 days. All studies were at high risk of bias for these outcomes due predominantly to large and unbalanced loss to follow up. The study<sup>166</sup> reporting data for 28 days was particularly high risk of bias due to a high risk of bias assessment in four out of five domains.

There was **very low certainty** evidence for an increase in infant time awake and alert (MD 38.3 minutes, 95% CI 18.2 to 58.4,  $p < 0.001$ ; 3 studies, 146 participants; Figure 3.11). There was substantial heterogeneity, with  $I^2$  of 74%, and the summary estimate was dominated by one study (with the highest number of domains assessed as high risk of bias). If this study were excluded, there would be no evidence for a difference in time spent awake and alert (mean difference of -15.2 minutes, 95% CI -64 to 33.7, 2 studies, 88 participants).

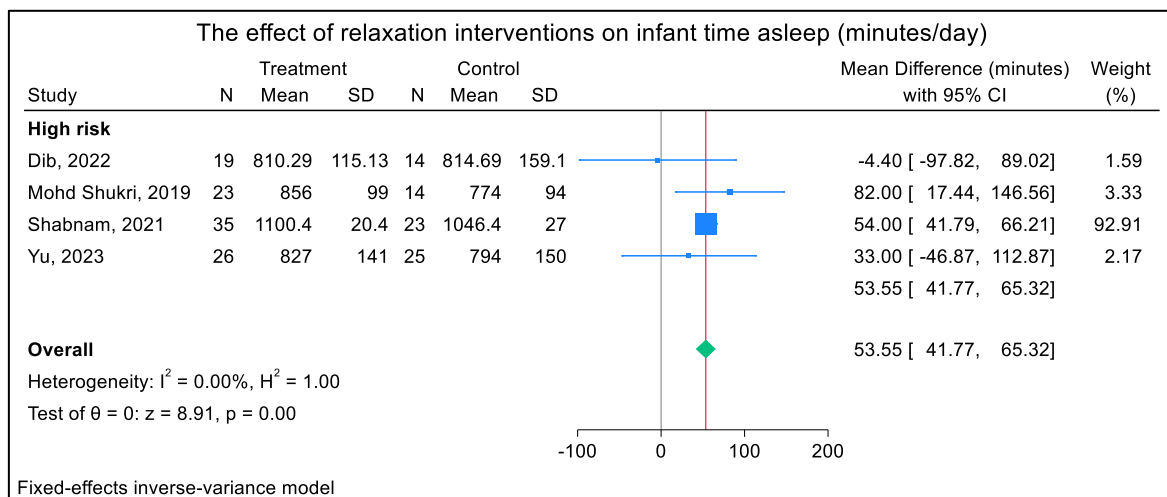
**Figure 3.11: Forest plot for infant time spent awake/calm, grouped by risk of bias assessment**



The GRADE assessment for the pooled effect estimate was very low certainty (downgraded by two levels for risk of bias and one level for inconsistency).

There was **low certainty** evidence for a moderate increase in infant sleep (MD 53.6 minutes, 95% CI 41.8 to 65.3,  $p < 0.001$ ; 4 studies, 179 participants; Figure 3.12). When the dominant study with highest risk of bias was excluded, the effect estimate remained of similar size (MD of 47.6 minutes, 95% CI 3.4 to 91.8,  $p < 0.001$ , 3 studies, 121 participants).

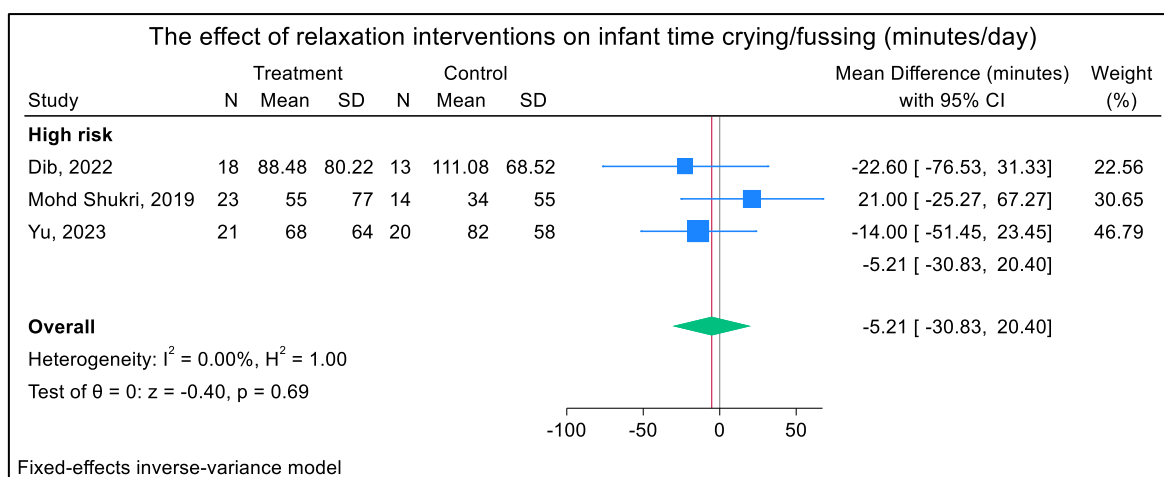
**Figure 3.12: Forest plot of infant time spent asleep, grouped by risk of bias assessment**



The GRADE assessment for the pooled effect estimate was low certainty (downgraded by two levels for risk of bias).

There was **low certainty** evidence for no change in infant crying time (MD of -5.2 minutes, 95% CI -30.8 to 20.4; 3 studies, 108 participants; Figure 3.13).

**Figure 3.13: Forest plot of infant time spent crying/fussing, grouped by risk of bias assessment**



The GRADE assessment for the pooled effect estimate was low certainty (downgraded by two levels for risk of bias).

### 3.3.4.6 Sensitivity analysis with use of random effects meta-analysis for primary outcomes

The effect estimates and resulting certainty of all the meta-analyses above were robust to the use of random effects technique apart from the time the infant spent awake and alert. For this outcome, random effects meta-analysis showed no significant effect (MD 11.4 minutes, 95% CI - 48.8 to 71.5, 3 studies, 146 participants). This was similar to the conclusion reported above when the heterogenous study was removed and casts significant doubt on this effect estimate, which is already classified as very low certainty.

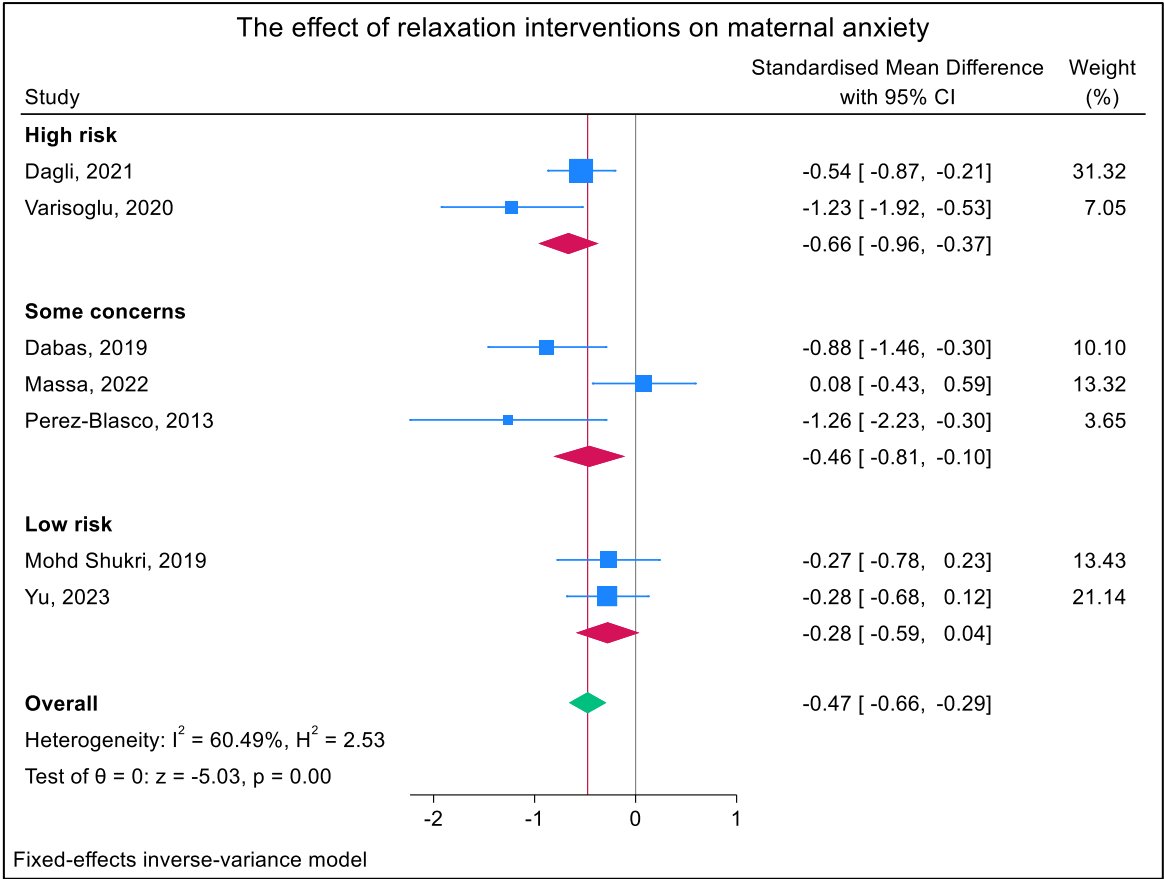
## 3.3.5 Secondary Outcomes

### 3.3.5.1 Maternal Mental Health

#### 3.3.5.1.1 Maternal Anxiety

There was **moderate certainty** evidence of a reduction in maternal anxiety (SMD -0.47, 95% CI - 0.66 to -0.29,  $p < 0.0001$ ; 7 studies, 483 participants; Figure 3.14). This is a reduction in anxiety score of 0.47 standard deviations, which is considered a small effect size<sup>177</sup>. There was substantial statistical heterogeneity ( $I^2 = 61\%$ ).

Figure 3.14: Forest plot for maternal anxiety score, grouped by risk of bias assessment



The GRADE assessment for the pooled effect estimate was moderate certainty (downgraded by one level for risk of bias).

These studies<sup>162,164,165,169,172,174,200</sup> measured maternal anxiety on four different psychometric scales. These were the Perinatal Anxiety Screening Scale<sup>200</sup>, Beck Anxiety Index<sup>162,172</sup>, the Depression, Anxiety and Stress (DASS 21) anxiety subscale<sup>169</sup> and the Spielberger State-Trait Anxiety Index<sup>164,165,174</sup>. The assessment timepoint ranged from within the two weeks after birth to around a year after birth.

Interventions were a lactation-specific guided relaxation<sup>162,172</sup>, a mindfulness app<sup>164</sup>, instrumental music<sup>165,174</sup>, a yoga breathing intervention<sup>200</sup> and mindfulness training<sup>169</sup>. Two studies were at high risk of bias for this outcome due to inadequate allocation concealment and results selection<sup>174</sup>; and non-randomised design<sup>165</sup>. Three studies<sup>164,169,200</sup> were classified as some concern over risk of bias due to the inherent subjectivity of self-reported anxiety in unblinded studies. The non-randomised crossover study<sup>165</sup> could only be included on an unpaired basis. As can be seen in Figure 3.14,

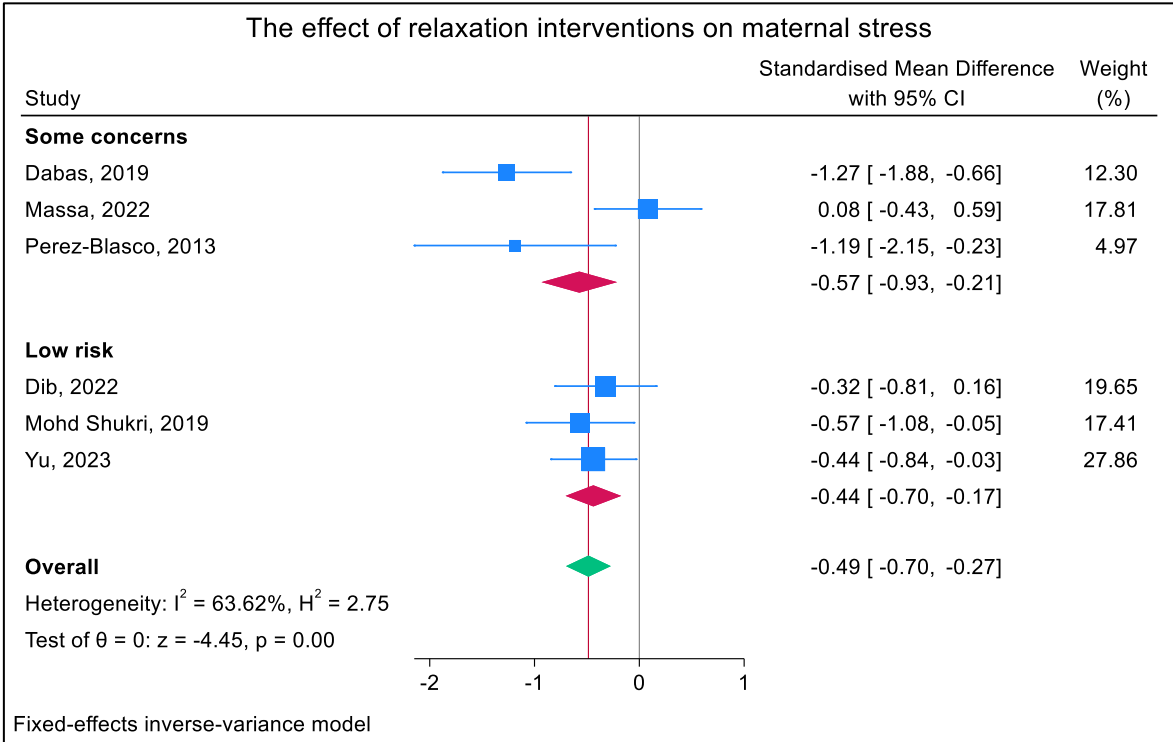
although the confidence intervals for all risk of bias subgroups included the overall effect estimate, there was a trend for a smaller effect estimate in lower risk studies.

One further study using the Symptom Checklist-90-R anxiety subscale after relaxation training could not be included in meta-analysis because of insufficient detail in outcome reporting<sup>201</sup>; it showed no difference after relaxation training (38 participants) and was classified as high risk of bias due to non-randomised design.

### 3.3.5.1.2 Maternal stress

There was **high certainty** evidence of a reduction in maternal stress (SMD -0.49, 95% CI -0.70 to -0.27,  $p < 0.0001$ ; 6 studies, 355 participants; Figure 3.15). This is a reduction in anxiety score of 0.49 standard deviations, which is considered a small effect size<sup>177</sup>. There was substantial statistical heterogeneity ( $I^2 = 64\%$ ).

**Figure 3.15: Forest plot for maternal stress score, grouped by risk of bias assessment**



These studies<sup>162–164,169,172,200</sup> measured maternal stress on three psychometric scales. These were the Perceived Stress Scale<sup>162,163,172</sup>, The NICU specific form of the Perceived Stress Scale<sup>164,200</sup>, and

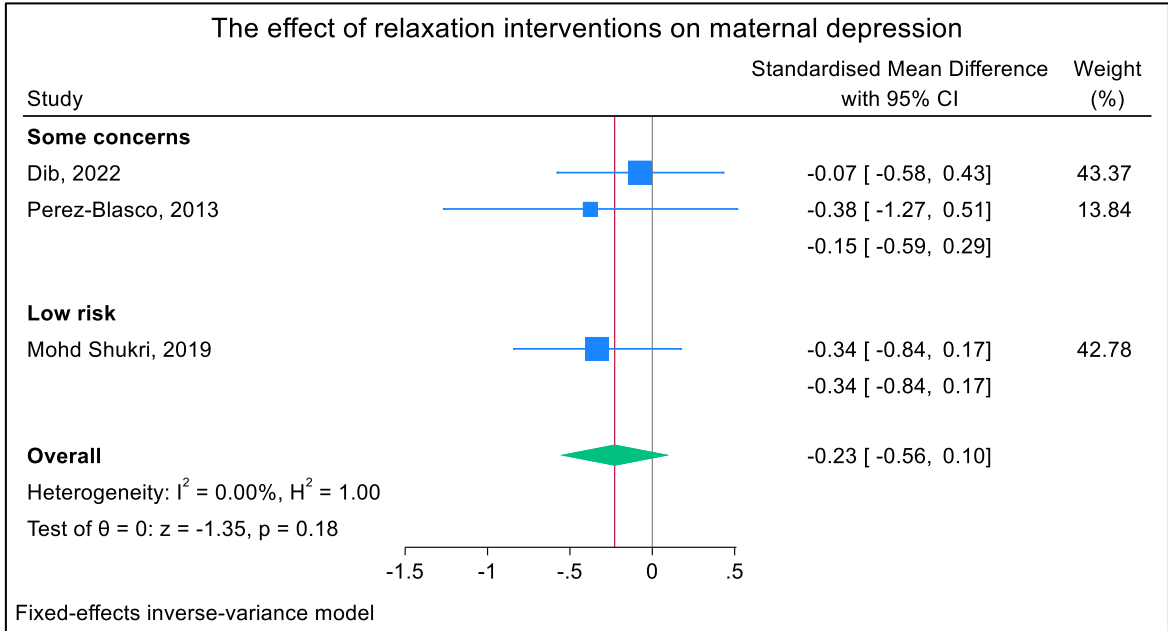
the Depression, Anxiety and Stress (DASS 21) stress subscale<sup>169</sup>. Three studies were classified as some risk of bias due to the inherent subjectivity of self-reported stress in unblinded studies.

One further study<sup>175</sup> reported stress with the Perceived Stress Scale but this could not be compared between intervention and control arms because of the crossover design and the timepoint of measurements, so could not be included.

### 3.3.5.1.3 Maternal Depression

There was **low certainty** evidence for no change in maternal depression (SMD -0.23, 95% CI -0.56 to 0.10; 3 studies, 143 participants; Figure 3.16).

**Figure 3.16: Forest plot for maternal depression, grouped by risk of bias assessment**



The GRADE assessment for the pooled effect estimate was low certainty (downgraded by one level for risk of bias and one level for imprecision).

These studies<sup>163,169,172</sup> reported an assessment of maternal depression using the Edinburgh Postnatal Depression Scale (EPDS)<sup>163,172</sup> and the Depression, Anxiety and Stress (DASS 21) depression subscale<sup>169</sup>. This outcome was classified as some concerns for risk of bias in two studies<sup>163,169</sup> due to the inherent subjectivity of self-reported depression in an unblinded study, and due to missing data.

Two further studies could not be included in meta-analysis due to the format of reporting of results or insufficient detail; neither showed a difference in depression measured with the EPDS (median difference -1, 95% CI -5 to 2.5<sup>164</sup>), or the Symptom Checklist-90-R depression subscale (mean difference 0.1<sup>201</sup>).

### 3.3.5.2 *Perception of Relaxation*

There was **low certainty** evidence for an increase in maternal perceived relaxation in a single study. The study<sup>108</sup> used a visual assessment scale (VAS) for participants to rate their feeling of relaxation before and after listening to a lactation-specific relaxation, music or control, in a crossover RCT with lactating mothers of healthy infants in the community, using a scale of 0 to 10.

The mean difference in VAS score was significantly higher in the guided relaxation arm compared to the control arm (MD 1.1, 95% CI 0.43 to 1.7,  $p < 0.01$ , 1 study, 20 participants), and in the music arm compared to control (MD 1.1, 95% CI 0.36 to 1.85,  $p < 0.05$ , 1 study, 20 participants). The study had some concerns over risk of bias due to the inherent subjectivity of relaxation self-assessment.

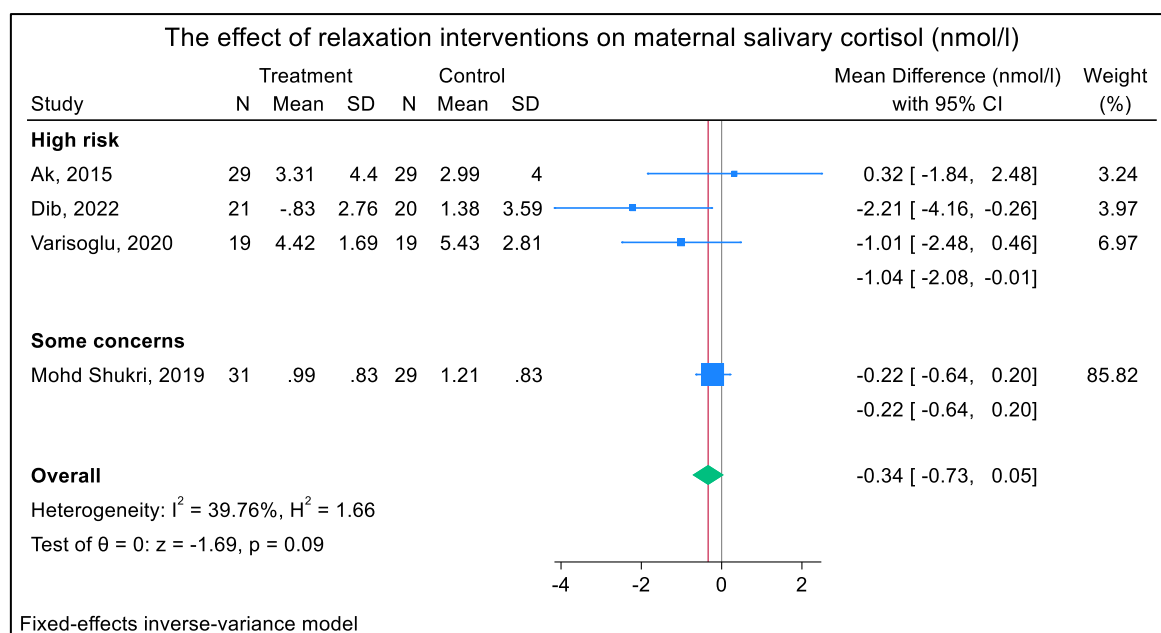
The GRADE assessment for the effect estimate was low certainty (downgraded by one level for risk of bias and one level for imprecision).

### 3.3.5.3 *Physiological markers of stress*

#### 3.3.5.3.1 *Salivary Cortisol*

There was **very low certainty** evidence for no change in maternal salivary cortisol after an episode of relaxation (MD -0.34 nmol/L, 95% CI -0.73 to 0.05; 4 studies, 168 participants; Figure 3.17). Heterogeneity did not exceed the pre-specified significant threshold ( $I^2$  of 40%).

**Figure 3.17: Forest plot for salivary cortisol, according to Risk of Bias assessment**



The GRADE assessment for the pooled effect estimate was very low certainty (downgraded by two levels for risk of bias and one level for imprecision).

These four studies measured maternal salivary cortisol<sup>163,172,174,175</sup> after an episode of relaxation intervention (or equivalent timepoint in the control group). In two studies this was after four episodes of exposure<sup>174,175</sup> to an instrumental music intervention in a NICU setting for mothers expressing milk. In two studies<sup>163,172</sup>, salivary cortisol was measured at six weeks after birth after several weeks' exposure to a lactation specific meditation for mothers breastfeeding healthy late preterm or term infants. One study with crossover design<sup>175</sup> was included here as unpaired data due to insufficient detail in the outcome report.

This outcome was classified as high risk of bias in three studies, due to inadequate allocation concealment and results selection<sup>174</sup>, inadequate washout in a crossover study<sup>175</sup> or the high level of missing data<sup>163</sup>. One study was classified as some concerns over risk of bias due to differences in the format of outcome reporting compared to the protocol<sup>172</sup>.

### 3.3.5.3.2 Vital signs

There was **moderate certainty** evidence for reduction in maternal blood pressure and heart rate, and increase in fingertip temperature in a single study<sup>108</sup>, measured before and after listening to a



lactation-specific relaxation. This was a crossover RCT with lactating mothers of healthy infants in the community. For example, for diastolic blood pressure MD -5.9 mmHg (95% CI -9.1 to -2.8,  $p < 0.01$ , 1 study, 20 participants). In the music arm of this study, only temperature was significantly different compared to control (MD 0.3 degrees centigrade, 95% CI 0.11 to 0.50,  $p < 0.01$ , 1 study, 20 participants).

The GRADE assessment for the effect estimate was moderate certainty (downgraded by one level for indirectness).

#### 3.3.5.4 *Breastfeeding self-efficacy*

There was **low certainty** evidence for no difference in breastfeeding self-efficacy in a single study<sup>164</sup>, using the Breastfeeding Self-Efficacy Scale short form for NICU after a week of recommended daily use of a mindfulness app (median difference 0, 95% CI -7.5 to 5, 1 study, 60 participants). This outcome is classified at high risk of bias due to missing data and the subjective nature of the measurement.

The GRADE assessment for the effect estimate was low certainty (downgraded by one level for risk of bias and one level for imprecision).

#### 3.3.5.5 *Frequency of breastfeeding or expressing*

There was **very low certainty** evidence for an increase in breastfeeding frequency in a single study<sup>163</sup> after provision of a guided relaxation and visualisation recording to mothers of late preterm and early term infants for about four weeks (MD 3.9 per day, 95% CI 1.13 to 6.67, 1 study, 18 participants). This outcome was at high risk of bias due to the level of missing data.

The GRADE assessment for the effect estimate was very low certainty (downgraded by one level for risk of bias and one level for imprecision).

There was **low certainty** evidence for no change in expressing frequency in a single study<sup>164</sup>, measured on day nine after birth after provision of a mindfulness app in the NICU setting (median

difference 1, 95% CI -1 to 1.5, 1 study, 54 participants). This outcome was classified as some concerns for risk of bias due to missing data.

The GRADE assessment for the effect estimate was low certainty (downgraded by one level for risk of bias and one level for imprecision).

#### 3.3.5.6 *Time spent breastfeeding*

There was **very low certainty** evidence for an increase in time spent breastfeeding in a single study<sup>166</sup> after use of instrumental music for low birth weight infants for four weeks (MD 124.9 minutes over 28 days, 95% CI 103.3 to 146.6, 1 study, 58 participants). This outcome was classified as high risk of bias due to missing data and unsatisfactory method of measurement.

The GRADE assessment for the effect estimate was very low certainty (downgraded by two levels for risk of bias and one level for imprecision).

#### 3.3.5.7 *Colostrum characteristics*

There was **very low certainty** for a reduction in the time to secretory activation (“lactogenesis II” or mature milk “coming in”) in a single study<sup>176</sup> (MD -1.1 days, 95% CI -1.68 to -0.60, 1 study, 62 participants). This study used relaxing flute music for 30 minutes twice a day. This outcome was classified as high risk of bias due to possible reporting selection and missing data.

The GRADE assessment for the effect estimate was very low certainty (downgraded by two levels for risk of bias and one level for imprecision).

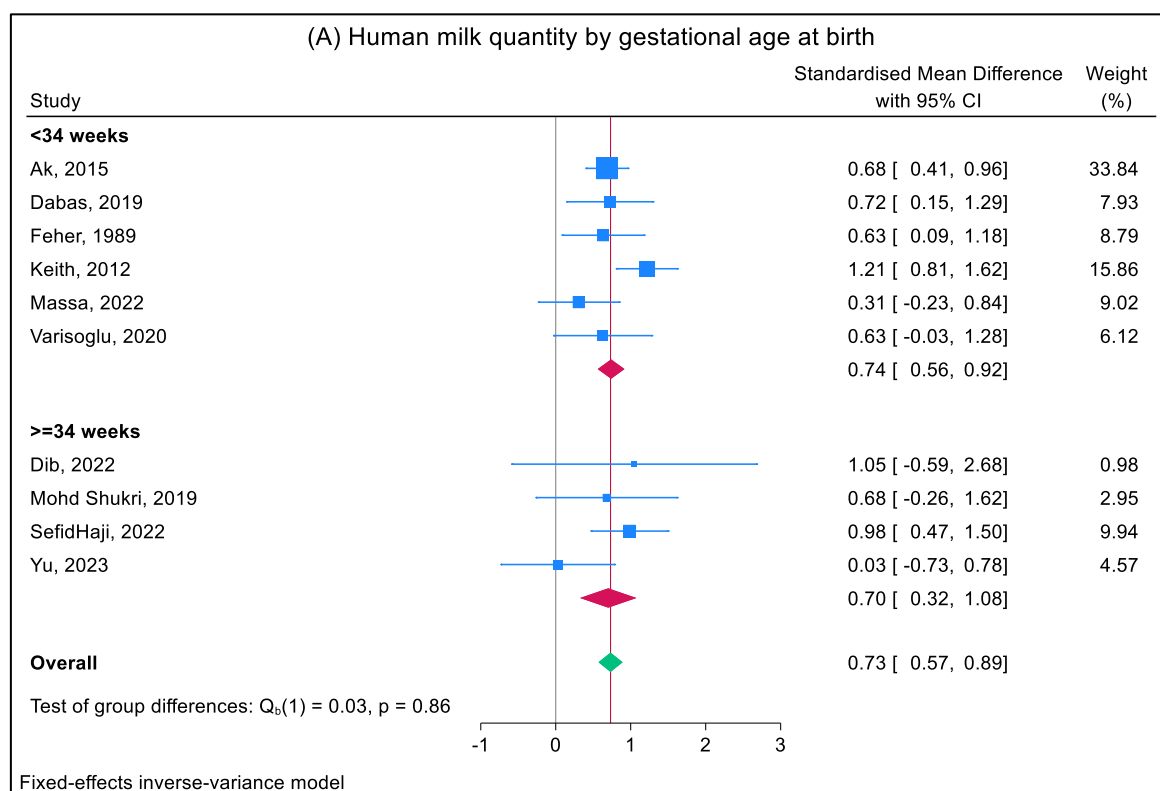
There was **very low certainty** for an increase in early colostrum quantity in a single study<sup>199</sup> using a calming song in the delivery room (RR of the highest ‘colostrum score’ of 1.58, 95% CI 1.24 to 2.00, 1 study, 304 participants). The highest score meant that three or more drops of colostrum were obtained after a single compression after the first breastfeed and within 2 hours of birth. There were some concerns over risk of bias due to concerns with unequal measurement timing between allocation arms.

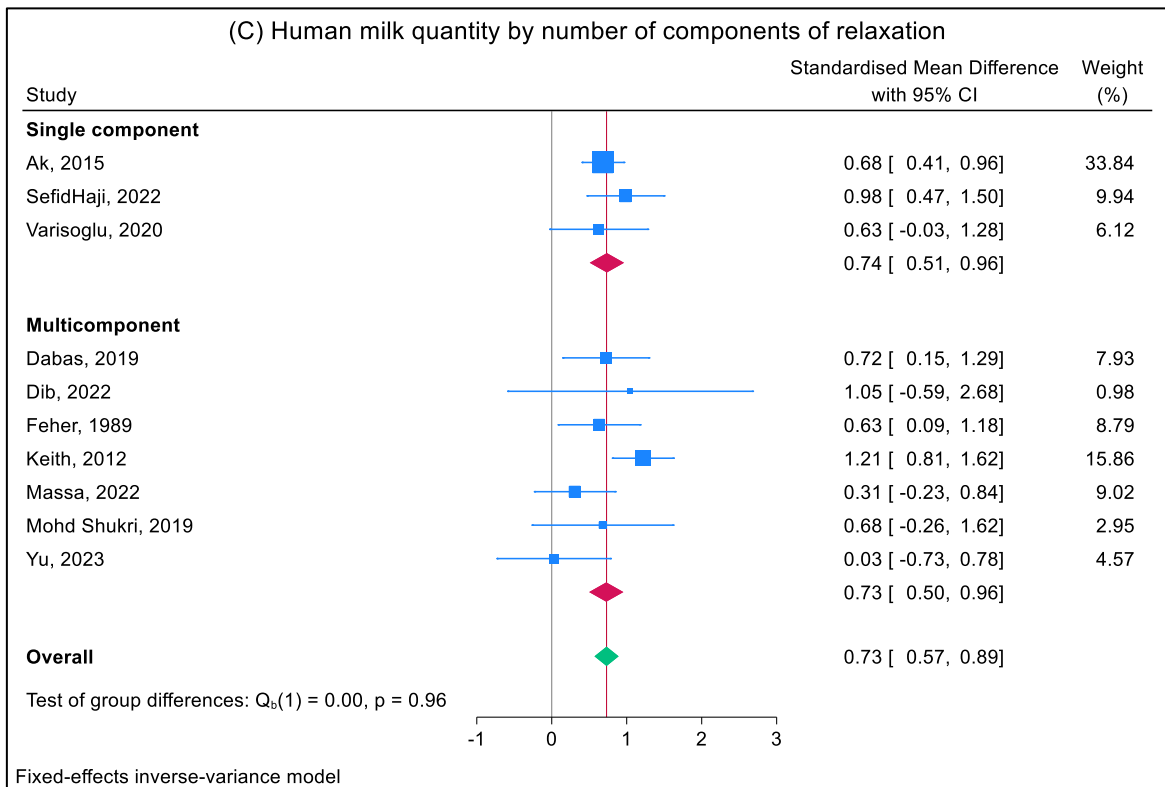
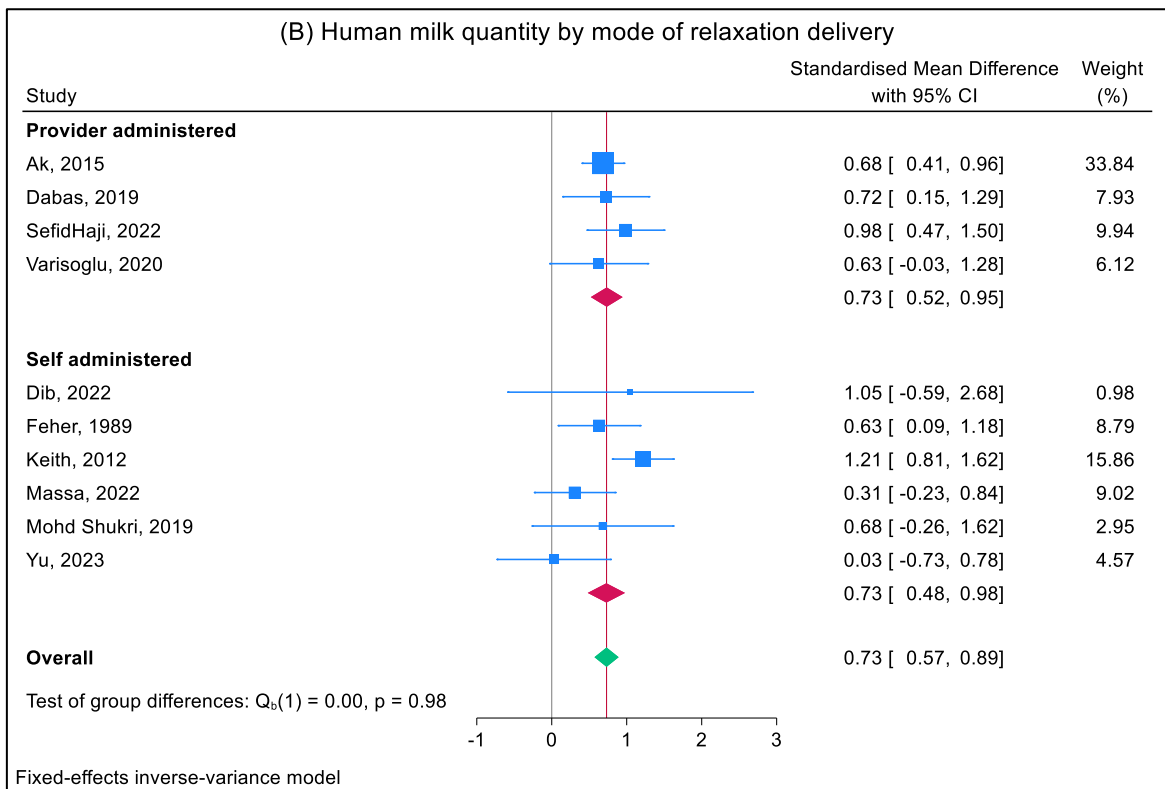
The GRADE assessment for the effect estimate was very low certainty (downgraded by one level for each of risk of bias, imprecision and indirectness).

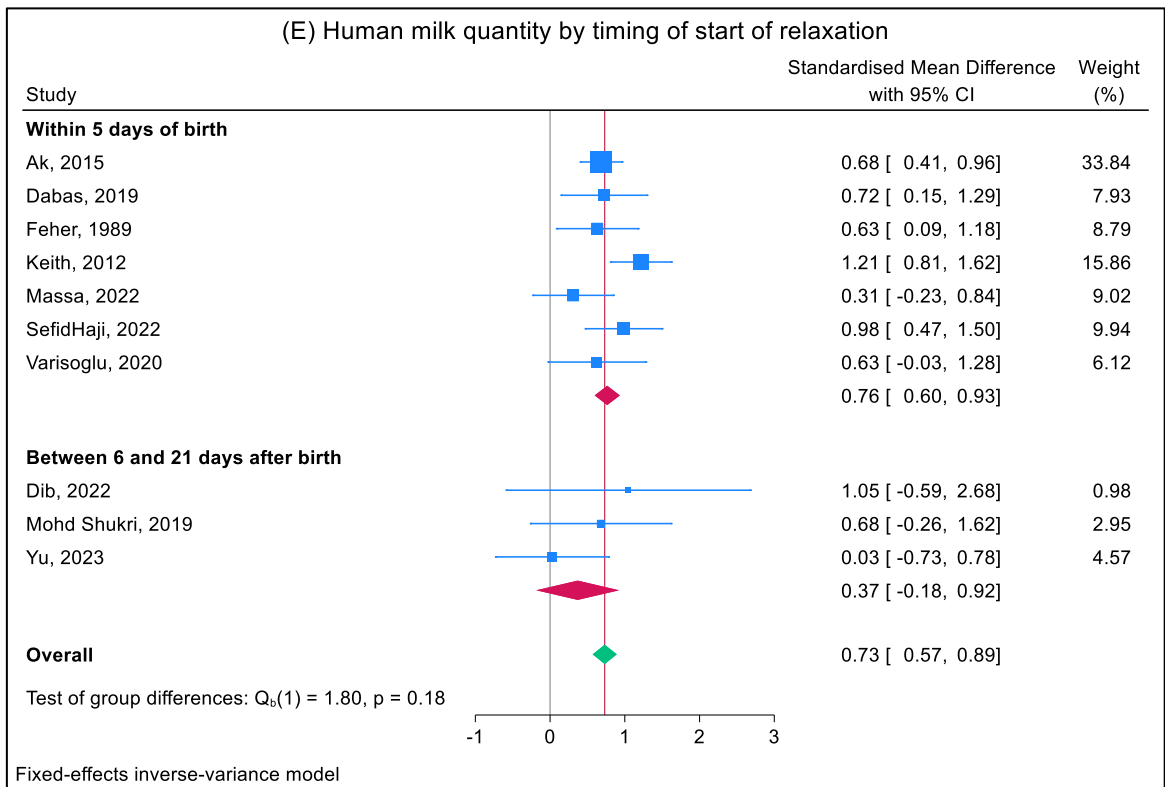
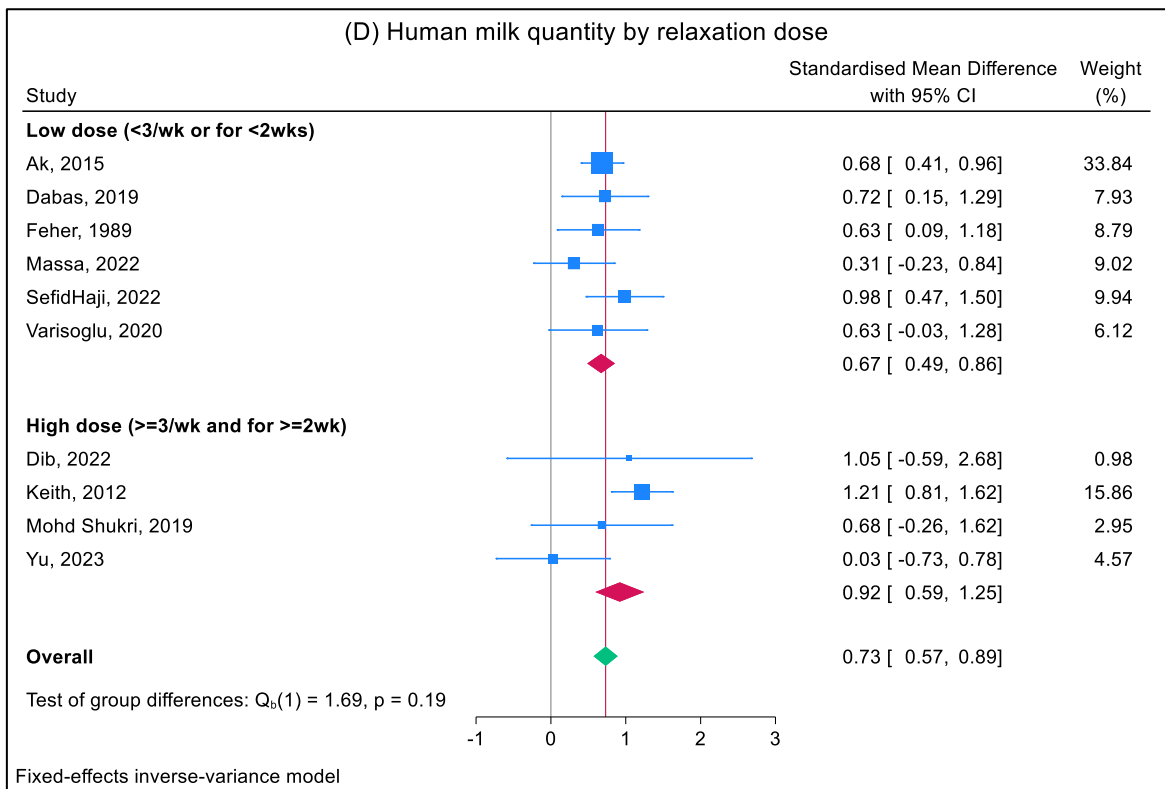
### 3.3.6 Subgroups

Breastmilk quantity was the only outcome with sufficient number of studies to explore sub-group effects. No significant differences in the pooled effect estimate were seen according to the nature of the relaxation intervention (complexity, timing of onset, dose and whether self-administered or not) or the gestational age at birth when assessed in two categories (Figure 3.18). For example, the SMD for infants born <34 weeks post-menstrual age (PMA) was 0.74 (95% CI 0.56 to 0.92) and for infants born ≥34 weeks' PMA this was 0.70 (95% CI 0.32 to 1.08; panel A; p=0.86). There were trends for a higher effect estimate for studies with higher 'dose' of relaxation and with an earlier start after birth (panel D and E; both p=0.2).

**Figure 3.18: Forest plots for sub-group analysis of breastmilk quantity**







### 3.3.7 Summary of evidence

Table 3.5 shows the summary of findings table for high and moderate certainty outcomes presented above, with provision of indicative examples of real life absolute and relative effects. Table 3.6 summarises low and very low certainty outcomes.

**Table 3.5: Summary of findings (high or moderate certainty)**

| Outcome                                      | Number of participants (studies) | Without relaxation <sup>2</sup>                                        | With relaxation (95% CI)                               | Effect estimate (95% CI)            | Certainty (downgrade reason)     | Comment                                         |
|----------------------------------------------|----------------------------------|------------------------------------------------------------------------|--------------------------------------------------------|-------------------------------------|----------------------------------|-------------------------------------------------|
| <b>Breastmilk and breastfeeding outcomes</b> |                                  |                                                                        |                                                        |                                     |                                  |                                                 |
| <b>Breastmilk quantity</b>                   | 469 (10 studies)                 | <b>611 g/day</b> (LPT/term baby at 2-3 months old)                     | <b>123 g higher</b> (96 to 150g higher)                | SMD: 0.73 (0.57 to 0.89)            | Moderate (1 level: risk of bias) | Clinically significant, moderate increase.      |
|                                              |                                  | <b>396 mL/day</b> (VPT, expressed milk yield at 2-3 weeks after birth) | <b>252 mL higher<sup>3</sup></b> (197 to 307ml higher) |                                     |                                  |                                                 |
| <b>Milk carbohydrate</b>                     | 139 (2 studies)                  | <b>42 g/day<sup>4</sup></b> (2-3 months after LPT/term birth)          | <b>0.9 g more</b> (0.1 to 1.8g more)                   | MD: 0.15 g/100 mL (0.01 to 0.29)    | Moderate (1 level: risk of bias) | Small increase, uncertain clinical significance |
| <b>Milk energy</b>                           | 139 (2 studies)                  | <b>400 kcal/day<sup>5</sup></b> (2-3 months after LPT/term birth)      | <b>11.2 kcal more</b> (0.6 to 21.8 kcal more)          | MD: 1.84 kcal/100 mL (0.10 to 3.57) | Moderate (1 level: risk of bias) | Small increase, uncertain clinical significance |
| <b>Milk protein</b>                          | 205 (3 studies)                  | <b>6 g/day<sup>6</sup></b> (2-3 months after LPT/term birth)           | No change                                              | MD: 0.00 g/100 mL (0.00 to 0.00)    | High                             | No change                                       |

<sup>2</sup> pooled results from control groups matching case description

<sup>3</sup> Expressed milk quantity has a higher variability than breastfeeding intake, thus the higher absolute effect estimate in this population

<sup>4</sup> Combining pooled control milk intake of 611g per day with pooled control milk carbohydrate of 6.8 g/100mL. 1ml and 1g of human milk are near equivalent

<sup>5</sup> Combining pooled control milk intake of 611g per day with pooled control milk energy of 65 kcal/100ml

<sup>6</sup> Combining pooled control milk intake of 611g per day with pooled control milk protein of 0.9g/100mL

| Outcome                                                  | Number of participants (studies) | Without relaxation <sup>2</sup>                                     | With relaxation (95% CI)                                                                                 | Effect estimate (95% CI)            | Certainty (downgrade reason)      | Comment                                         |
|----------------------------------------------------------|----------------------------------|---------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|-------------------------------------|-----------------------------------|-------------------------------------------------|
| <b>Mental health and physiology (parent)</b>             |                                  |                                                                     |                                                                                                          |                                     |                                   |                                                 |
| <b>Vital signs – example is diastolic blood pressure</b> | 20 (1 study)                     | <b>80 mmHg*</b>                                                     | <b>6 mmHg lower</b> (3 to 9 mmHg lower)                                                                  | MD: -5.9 mmHg (-9.1 to -2.8)        | Moderate (1 level: indirectness)  | Small decrease, uncertain clinical significance |
| <b>Stress</b>                                            | 355 (6 studies)                  | <b>Perceived Stress Scale 17</b> (2-3 months after LPT/ term birth) | <b>Reduce to 14</b> (reduce to 13 to 16)                                                                 | SMD: -0.49 (-0.70 to -0.27)         | High                              | Clinically significant, small decrease          |
| <b>Anxiety</b>                                           | 399 (7 studies)                  | <b>Beck Anxiety Index of 8</b> (2-3 months after LPT/ term birth)   | <b>Reduce to 5</b> (reduce to 3 to 6)                                                                    | SMD: -0.47 (-0.66 to -0.29)         | Moderate (1 level: risk of bias)  | Small decrease, uncertain clinical significance |
| <b>Growth and behaviour outcomes (infant)</b>            |                                  |                                                                     |                                                                                                          |                                     |                                   |                                                 |
| <b>Weight</b>                                            | 226 (3 studies)                  | Infant on <b>50<sup>th</sup> centile*</b>                           | <b>Increase to 69<sup>th</sup> centile</b> (62 <sup>nd</sup> to 76 <sup>th</sup> )                       | MD: 0.51 SDS change (0.30 to 0.72)  | High                              | Clinically significant, moderate increase       |
|                                                          |                                  | Infant on <b>5<sup>th</sup> centile</b>                             | <b>Increase to 13<sup>th</sup> centile</b> (9 <sup>th</sup> to 18 <sup>th</sup> )                        |                                     |                                   |                                                 |
| <b>Length</b>                                            | 226 (3 studies)                  | Infant on <b>50<sup>th</sup> centile*</b>                           | <b>Increase to 52<sup>nd</sup> centile</b> (reduce to 42 <sup>nd</sup> to increase to 61 <sup>st</sup> ) | MD: 0.04 SDS change (-0.21 to 0.29) | Moderate (1 level: inconsistency) | No change                                       |
|                                                          |                                  | Infant on <b>5<sup>th</sup> centile</b>                             | <b>Increase to 6<sup>th</sup> centile</b> (reduce to 3 <sup>rd</sup> to increase to 9 <sup>th</sup> )    |                                     |                                   |                                                 |

SMD = standardised mean difference; RR = risk ratio. MD = mean difference. \*Population averages. LPT = late preterm. VPT = very preterm

**Table 3.6: Summary of findings (low or very low certainty)**

| Outcome                                               | Number of participants (studies) | Effect estimate (95% CI)           | Certainty (downgrade reason)                           | Comment                                              |
|-------------------------------------------------------|----------------------------------|------------------------------------|--------------------------------------------------------|------------------------------------------------------|
| <b>Breastmilk and breastfeeding outcomes</b>          |                                  |                                    |                                                        |                                                      |
| <b>Exclusive breastmilk</b>                           | 651 (2 studies)                  | RR: 0.99 (0.90 to 1.09)            | Low (1 level: risk of bias; imprecision)               | No change                                            |
| <b>Any breastmilk</b>                                 | 47 (1 study)                     | RR: 1.18 (0.89 to 1.58)            | Low (1 level: risk of bias; imprecision)               | No change                                            |
| <b>Milk fat</b>                                       | 387 (5 studies)                  | SMD: 0.17 (-0.04 to 0.37)          | Low (1 level: risk of bias; imprecision)               | No change                                            |
| <b>Milk cortisol</b>                                  | 63 (1 study)                     | MD: -44.5 % (-76.1 to -12.9)       | Low (1 level: risk of bias; imprecision)               | Moderate decrease of uncertain clinical significance |
| <b>Expressing frequency</b>                           | 51 (1 study)                     | MD: 1 per day (-1 to 1.5)          | Low (1 level: risk of bias; imprecision)               | No change                                            |
| <b>Breastfeeding self-efficacy</b>                    | 60 (1 study)                     | Median Difference: 0* (-7.5 to 5)  | Low (1 level: risk of bias; imprecision)               | No change                                            |
| <b>Time spent breastfeeding</b>                       | 58 (1 study)                     | MD: 124.9 minutes (103.3 to 146.6) | Very low (2 levels: risk of bias; 1 level imprecision) | Large increase of uncertain clinical significance    |
| <b>Breastfeeding frequency</b>                        | 18 (1 study)                     | MD: 3.9 per day (1.1 to 6.7)       | Very low (2 levels: risk of bias; 1 level imprecision) | Small increase of possible clinical significance     |
| <b>Time to secretory activation (lactogenesis II)</b> | 62 (1 study)                     | MD: -1.1 days (-1.7 to -0.6)       | Very low (2 levels: risk of bias; 1 level imprecision) | Moderate decrease of possible clinical significance  |
| <b>Colostrum score</b>                                | 304 (1 study)                    | RR 1.6 (1.2 to 2.0)                | Very low (2 levels: risk of bias; 1 level imprecision) | Small increase of minimal clinical significance      |
| <b>Growth and behaviour outcomes (infant)</b>         |                                  |                                    |                                                        |                                                      |
| <b>Head circumference</b>                             | 58 (1 study)                     | MD: 0.27 cm (-0.53 to 1.07)        | Very low (2 levels: risk of bias; 1 level imprecision) | No change                                            |
| <b>Fat free mass</b>                                  | 29 (1 study)                     | MD: 0.5 kg (-0.06 to 1.06)         | Very low (2 levels: risk of bias; 1 level imprecision) | No change                                            |
| <b>Time sleeping</b>                                  | 179 (4 studies)                  | MD: 53.6 minutes (41.8 to 65.3)    | Low (2 levels: risk of bias)                           | Clinically significant, moderate increase            |
| <b>Time crying/fussing</b>                            | 108 (3 studies)                  | MD: -5.2 minutes (-30.8 to 20.4)   | Low (2 levels: risk of bias)                           | No change                                            |



| Outcome                                                                    | Number of participants (studies) | Effect estimate (95% CI)         | Certainty (downgrade reason)                             | Comment                                                       |
|----------------------------------------------------------------------------|----------------------------------|----------------------------------|----------------------------------------------------------|---------------------------------------------------------------|
| Time awake and alert                                                       | 146 (3 studies)                  | MD: 38.3 minutes (18.2 to 58.4)  | Very low (2 levels: risk of bias; 1 level inconsistency) | Small increase of possible clinical significance <sup>7</sup> |
| <b>Mental health and stress related outcomes (mother/lactating parent)</b> |                                  |                                  |                                                          |                                                               |
| Perceived relaxation                                                       | 20 (1 study)                     | MD: 1.1 (0.43 to 1.7)            | Low (1 level: risk of bias; 1 level imprecision)         | Small increase of possible clinical significance              |
| Depression                                                                 | 143 (3 studies)                  | SMD: -0.23 (-0.56 to 0.10)       | Low (1 level: risk of bias; imprecision)                 | No change                                                     |
| Salivary cortisol                                                          | 168 (4 studies)                  | MD: -0.34 nmol/L (-0.73 to 0.05) | Very low (2 levels: risk of bias; 1 level imprecision)   | No change                                                     |

SMD = standardised mean difference; RR = risk ratio. MD = mean difference. \*Breastfeeding self-efficacy Scale (short form for NICU)

### 3.4 Discussion

This systematic review update has added thirteen new studies and more than 1600 more participants to the previous review from 2016, strengthening potential conclusions and facilitating meta-analyses for the first time. All five studies reporting outcomes that were assessed as low risk of bias were published since 2019<sup>162,163,167,172,197</sup>, which increases the certainty of the review conclusions. However, many effect estimates were still assessed as low or very low certainty due to difficulties with risk of bias and imprecision.

Meta-analysis of current evidence provides high certainty that provision of relaxation reduces stress in the mother; increases infant weight gain by a moderate amount (when provided to directly breastfeeding mothers/parents); and does not change milk protein content.

There is moderate certainty that provision of relaxation increases milk quantity by a moderate, clinically significant amount; reduces maternal anxiety by a small amount; increases milk

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<sup>7</sup> Not robust to sensitivity analysis

carbohydrate and energy by a small amount; induces a physiological 'relaxation response' (for example, reduced blood pressure); and does not change infant length.

There is low certainty evidence that relaxation increases the time spent sleeping by the infant, decreases immediate milk cortisol and induces a perception of relaxation. There is low certainty evidence that relaxation has no impact on provision of any or exclusive breastmilk, breastmilk fat, maternal depression, time spent crying by the infant, frequency of milk expression and breastfeeding self-efficacy.

Qualitative evidence is a useful addition to quantitative meta-analysis when cognitive experience is involved. One publication has described the impact of mindfulness training on NICU parents, with reports that this helped some parents to calm the chaotic feelings generated by their infant's condition, reduce the distress associated with guilt, fear and lack of control, and focus on connection with the infant<sup>206</sup>. Numerical measures of stress, depression and anxiety may not capture this change in parental experience and may therefore underestimate the value of relaxation interventions for parents.

Evidence synthesis is difficult in the context of the very high level of heterogeneity represented within the field of relaxation interventions. The studies were from a wide variety of countries (China, India, Iran, Malaysia, Spain, Thailand, Turkey, UK and USA). Relaxation techniques are more accepted and commonly used in some cultures; for example several studies commented on using traditional instrumental music that had specific cultural congruence. The congruence of the intervention for the specific population is likely to affect adherence; for example, parents have reported that mindfulness tracks that are designed for people experiencing normal pregnancy and birth may be inappropriate and distressing for those experience traumatic births and with sick and preterm infants, as they often emphasise trusting your body or use imagery of a healthy breastfeeding baby<sup>2</sup>.

There may also be cultural factors relating to the postnatal environment that enhance or decrease adherence. For example, whether there is an expectation of the mother resting for several weeks

after birth with household tasks taken on by other family members, which is more common in East Asian countries such as China and Malaysia.

Personal factors, such as the patient's previous experience with relaxation, their expectations and their ability to form vivid mental imagery can affect the efficacy of therapeutic relaxation<sup>95,107</sup>. Some people feel that interventions such as meditation and mindfulness are 'not for them' and even if they enter trials they are less likely to engage with these practices<sup>206</sup>.

The potential impact of these factors are difficult to assess using statistical techniques as each study is likely to have a unique cultural context and relevant information about the postnatal environment, cultural attitudes to relaxation and modifications to the intervention are infrequently reported.

Most studies used either lactation-specific guided relaxation or instrumental music, but some used a variety of mindfulness and relaxation training. Some interventions were delivered or recommended for only hours or days, whereas for others this was weeks or months. The population was a mixture of parents directly breastfeeding in the community and those expressing milk for sick and preterm infants in the NICU. Parents of infants in a neonatal unit are likely to have higher baseline anxiety and distress than those in the community<sup>67,207</sup>. Expressing breastmilk for preterm and sick infants is a very different process to directly breastfeeding a healthy infant and in particular, the variability of the quantity of milk expressed is much higher than when directly breastfeeding a healthy infant.

It is surprising but reassuring that in the setting of such clinical heterogeneity, most analyses were not affected by substantial statistical heterogeneity. There was also no evidence of subgroup differences in the effect of relaxation on milk quantity by factors such as dose or infant gestation, but there were insufficient studies providing data for other outcomes to explore this fully and the number of categories within each subgroup was limited.

Although no significant difference was noted in the effect estimate by intervention dose at a study level, individual studies have reported a 'dose response' effect. However this is an association of

improved outcomes (breastmilk quantity<sup>164,202</sup>, maternal stress<sup>162</sup>, weight gain<sup>162,163</sup> and breastfeeding frequency<sup>163</sup>) with increased adherence with the relaxation intervention, which is more prone to bias. Participants who have high intervention adherence are likely to be different to those with lower or no adherence, and to some participants in the control arm, in many other ways that affect lactation. One study found evidence of differences in the baseline characteristics of those with high and low adherence, and adjusted for them<sup>164</sup> but unmeasured confounding is likely to persist. These reported 'dose response' relationships should be viewed with caution, but merit further investigation.

In many of the included studies, the intervention was specifically targeted at the mother with use of headphones or because the intervention is recommended during expressing rather than breastfeeding where the mother and infant may be physically separated. However, it is likely that the infants were also exposed to the relaxation intervention in some studies and that this could mediate some of the effects seen; indeed one study specifically interpreted the results as due to the effect of music on the infant rather than the mother<sup>166</sup>.

Meta-analysis of music therapy for preterm infants shows an effect on oral feeding volumes and breastfeeding rates in some studies<sup>208</sup>. However, these studies used music as a positive reinforcement for sucking (pacifier activated devices); studies of simple background music targeted at the infant have shown mixed effects<sup>209</sup>. Outcomes such as infant growth and behaviour could be affected by either or both mother and infant being exposed to relaxation. It is unlikely that expressed milk quantity and milk composition would be substantially affected by infant exposure to relaxation.

Overall, the moderate to high certainty evidence of improvements in infant weight gain, breastmilk quantity and maternal mental health suggest that self-directed relaxation interventions can be offered to lactating parents. The lack of major potential harm from relaxation, and high acceptability to the general population, is further reason for confidence in this recommendation.

Further research would be helpful for populations with high risk of poor lactation outcomes, particularly those who are time poor and therefore need a greater certainty in the impact of investing time in relaxation interventions. This is particularly relevant to parents of infants admitted to NICU, due to the demanding and time consuming expressing regime recommended, as well as the need for travel between home and hospital. These parents also experience higher rates of stress and anxiety<sup>67</sup>.

Future studies in the area of relaxation and lactation should aim to integrate objective measures of effect with participant self-assessment (which is valid and important despite having an inherent subjectivity and therefore risk of bias in an unblinded setting). More qualitative data would also be useful on parents' cognitive experience of relaxation. Exploration of the value of relaxation to parents would be useful to quantify this aspect (i.e. do they like the intervention?).

More attention should also be paid in future work to high quality trial processes such as allocation concealment and pre-specified statistical analysis plans to avoid selection bias; these were present in some studies included in this meta-analysis, reducing the strength of the evidence. Crossover studies may not be appropriate in this setting unless parents are at a stable stage of lactation and the washout period is sufficient. Differential missing data was seen in several trials, with more loss to follow up in the control arm, particularly for outcomes involving high participant burden such as infant behaviour diaries and test weighing<sup>163,166,169,172</sup>, so attempts for pre-emptive mitigation for this issue should be made for future trials.

### **3.4.1 Impact of these findings on the randomised trial design**

Although the final search was performed in February 2023, earlier searches were performed at the beginning of the doctoral timeline. What was learned supported the RCT design as follows:

- Parallel group randomised design
- Choice of population – high risk for lactation and mental health difficulties, time poor

- Emphasis on including participants with experience of the most preterm birth, as they have the highest risk for poor lactation outcome
- Influenced the choice of intervention (as one existing recording has been used in multiple previous studies)
- Involvement of parents in the modification of the intervention for cultural congruence and acceptability
- Involvement of parents in the choice of research outcomes rather than simply following what prior studies have reported (as will be reported in Chapter 4)
- Conducting the trial within a Clinical Trials Unit, thus ensuring high quality processes for randomisation, allocation concealment and trial management processes
- Pre-emptive risk assessment including potential for unbalanced missing data between allocation arms
- Pre-specified statistical analysis plan, with reference to limitation of multiple comparisons, use of sensitivity analyses and exploration of adherence effects
- 24-hour logs of expressing quantity rather than single sessions
- Opportunity for undirected qualitative feedback from participants on their experience of the intervention
- Quantitative assessment of participant's experience of the intervention (Likert scales)

Elements that were considered but could not be incorporated

- Blinding of the participants to the allocation arm
- Milk sampling (more objective outcome). The added logistical complexity of milk collection and analysis would have been too much for the doctoral resources, particularly because participants are often repatriated to local hospitals during the study period
- Infant growth measures (more objective outcome). The context of NICU management of growth after very preterm birth meant that it is extremely unlikely that relaxation could affect these outcomes. For example, donor breastmilk or infant formula is used to achieve

specific feed volumes where maternal milk is insufficient, and breastmilk is fortified to ensure macronutrient sufficiency or in response to impaired growth.

### **3.5 Learning from the process of systematic review**

A decision was made to update the 2016 review rather than re-search the literature, because this was felt to be the most efficient use of time and the 2016 review was focused on the same broad area as the thesis objectives.

However this did bring some limitations due to the decisions made to set eligibility criteria for the original review. The original review had excluded non English language studies, which was not felt to be appropriate given the highly international nature of studies in this area. Therefore this criterion was removed, however use of non-English search portals would have been needed to thoroughly search for non-English publications so this may still have limited the breadth of the search and potentially affected the conclusions of review and meta-analysis.

The original review had included non-randomised studies, which introduces higher risk of bias to the analysis. However, the two non-randomised studies included had minimal impact on the conclusions of the review and were only included<sup>165</sup> in one meta-analysis (of maternal anxiety). Finally, there were a large number of outcomes specified in the original review, including a long list of primary outcomes, and no pre-specified definitions for these outcomes.

The search terms for the original review were improved for the update review, in terms of breadth and use of specific keywords. If I were to start the systematic review process again, I would design eligibility criteria and search terms from scratch rather than using the 2016 review as a basis.

### **3.6 Summary**

This chapter has described the results of a systematic review of the literature surrounding relaxation for lactating mothers. High and moderate certainty evidence of improvement in maternal stress and anxiety, milk quantity, milk carbohydrate and energy, infant weight gain and physiological signs of a 'relaxation response' were demonstrated. The next chapter will outline the

programme of parent involvement work that took place to inform the design and oversight of a randomised trial of a relaxation intervention for mothers of very preterm infants.



# **Chapter 4: Parent Public Involvement (PPI) to design a randomised controlled trial of relaxation after very preterm birth**

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## **4.1 Chapter Overview**

This chapter describes the iterative process of collaboration with parents of preterm infants in order to plan, design and carry out a randomised controlled trial of relaxation for lactating mothers of very preterm infants. This is an example of Public Patient Involvement in research (PPI), whose theoretical underpinnings will be described further here. The influence of each stage of PPI work on the programme of work described in subsequent chapters will be discussed.

The research presented in this chapter has been published in The International Breastfeeding Journal<sup>2</sup>. I am the lead author of this paper. The co-authors are three doctoral supervisors, a colleague in the National Perinatal Epidemiology Unit with qualitative research expertise and a PPI representative. I carried out the research described and wrote the manuscript. The co-authors supported the design of the research and provided feedback on the manuscript. All co-authors have given permission to use relevant material from the published paper in this thesis. The published paper is provided as Appendix document B.1 for reference.

## **4.2 Background**

### **4.2.1 What is PPI?**

Patient and Public Involvement is the process of involving those who benefit from research in planning, performing and disseminating research. In a neonatal context (where the infant is unable to communicate in this way), the term patient covers the parents of a newborn as well as older children or adults who previously experienced neonatal care<sup>210</sup>. PPI at every stage of research is becoming more established<sup>211,212</sup> but brings with it a complex power dynamic and a need for specific

resource allocation at an early stage, often before funding applications are written<sup>213</sup>. Early-stage researchers, particularly students, may therefore encounter challenges to integrating effective PPI into research plans<sup>214</sup>.

Positive impacts of PPI include increasing trial recruitment or retention<sup>211</sup>, refining the research question to ensure it has maximum utility<sup>215</sup>, correcting bias and incorrect assumptions<sup>216</sup>, and pre-emptively identifying potential problems<sup>217</sup>. In addition, PPI can be viewed through a rights-based lens as a social good<sup>215</sup> and through a political lens as necessary for the accountability and legitimacy<sup>218</sup> of science. It is important to pay attention to potential discrimination in whose voices are heard, which will involve particular emphasis on involving marginalised groups<sup>215,219,220</sup>.

#### **4.2.2 Overview of PPI stages**

There were five major PPI stages at the planning and design stage of the trial (stage one to five), another stage supported ongoing trial management (stage six) and one further stage is in process for dissemination (stage seven). These stages are described in Table 4.1 and their relationship with the trial lifecycle is shown in Figure 4.1.

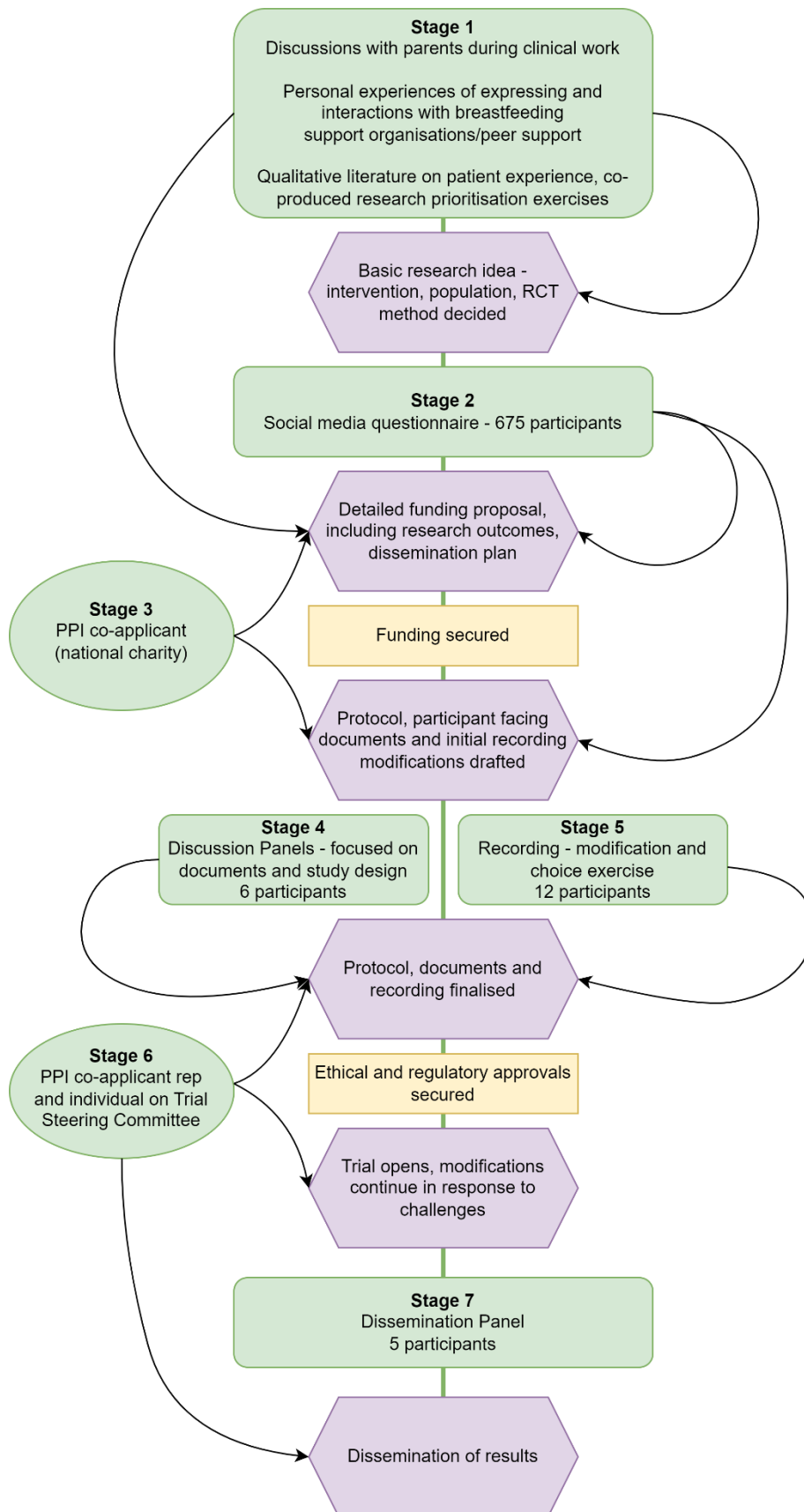
**Table 4.1: Description of PPI stages complete, ongoing and planned**

| Stage                                                       | Description                                                                                                                                                                                                                                 | Demographic characteristics                                                                                                                            |
|-------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>1. Informal PPI (complete)</b>                           | Informal discussions with families over ten years of neonatal practice; personal experiences with expressing breastmilk and breastfeeding support communities; published qualitative literature and partnership prioritisation exercises    | Varied family characteristics                                                                                                                          |
| <b>2. Online Questionnaire (complete)</b>                   | Online questionnaire with free text questions and prioritisation exercises. Advertised via Bliss (national charity for sick and preterm infants)                                                                                            | 675 contributors. 71% experienced birth before 32 weeks of pregnancy. 95% White ethnic background                                                      |
| <b>3. Charity Collaborator (complete)</b>                   | Bliss staff members involved in creation of funding application and RCT protocol. Note, continued involvement in later stages of steering committee and dissemination panel                                                                 | Two staff members                                                                                                                                      |
| <b>4. Online Panels (complete)</b>                          | Two evening online panels. Contributors were sent documents to review in advance. Discussion of trial design and documents encouraged. Role description provided in advance and payment offered as per INVOLVE guidance <sup>221</sup>      | Six contributors. Two from Black and other minority ethnic backgrounds. One was under 25 years old                                                     |
| <b>5. RCT Intervention Modification Exercise (complete)</b> | Contributors were sent two draft intervention recordings and focused questionnaires for feedback and recommendations. Role description provided in advance and payment offered as per INVOLVE guidance                                      | 12 contributors. Four from Black and other minority ethnic backgrounds. Two contributors with English as a second language. One was under 25 years old |
| <b>6. RCT Steering Committee (complete)</b>                 | PPI contributors on the Trial Steering Committee attended meetings and were consulted when difficulties occurred. Role description provided in advance and payment offered as per INVOLVE guidance.                                         | One parent and one Bliss staff member                                                                                                                  |
| <b>7. Dissemination Panel (ongoing)</b>                     | Individual and group meetings with trial participants and Bliss staff to discuss the meaning of results and create public facing dissemination materials. Role description provided in advance and payment offered as per INVOLVE guidance. | In addition to Bliss staff member, five contributors, three of whom are Black. All except one have degree level educational background                 |

*A version of this table has been previously published<sup>2</sup>*

Figure 4.1 shows that early PPI can influence the entire research cycle, and that later PPI stages can deepen and refine elements that have been raised in earlier, broader stages.

**Figure 4.1: Interaction of PPI stages with the research lifecycle**



*A version of this figure has been previously published<sup>2</sup>*

## **4.3 Integrated Methods and Results: Structure and influences of PPI**

### **4.3.1 Stage 1 – Informal PPI**

#### *4.3.1.1 Structure*

I am a neonatal clinician with a particular interest in lactation and I have listened to parents in newborn care units during clinical work over ten years of practice. I also have personal experience of expressing breastmilk for prolonged periods and involvement in breastfeeding support organisations and peer support networks as a service user and trustee.

Literature review of parental qualitative experience and consultation of co-produced prioritisation also falls into this stage (themes from these sources have been described in Chapter 2). These factors informed a basic understanding of parental experiences, priorities and values that could be used as a springboard for formal PPI planning and broad decisions on research design.

#### *4.3.1.2 Influence*

This informal PPI foundation influenced decisions about the intervention area (relaxation recording), the population (mothers of very preterm infants), the method (RCT) and the broad outcome set (relating to expressed milk volume and breastfeeding experiences).

### **4.3.2 Stage 2 – Online questionnaire**

#### *4.3.2.1 Structure*

An online questionnaire was advertised through social media channels of Bliss, a large charity supporting families with sick and preterm infants in the United Kingdom. Bliss provides parent and professional resources, individual support, national advocacy and supports research. The questionnaire was designed to elicit opinions from parents of preterm infants with experience of lactation on research priorities related to lactation, what outcomes are most important, potential practical and ethical difficulties of a trial and where parents obtain lactation information. No specific

target number of respondents had been pre-specified, however the questionnaire was closed after one week due to the overwhelming response.

Six hundred and seventy-five people responded to the questionnaire (respondents were not asked their gender but all had experience of lactation). Forty-seven percent had an infant born between 28 and 32 weeks of gestation. A quarter (24%) had an infant born at less than 28 weeks' gestation. Half (51%) had provided breastmilk to their infant for six months or less. A quarter (23%) had provided breastmilk for more than one year. 95% of respondents reported White ethnic background. The sample included 30 mothers from an ethnic minority background and 52 who were under 25 years old at the time of the preterm birth.

#### 4.3.2.2 *Influence – choice of outcomes*

The first key influence on the research design was in the choice of outcomes. When asked what questions they had about how to optimally express for and breastfeed a preterm baby, the most common question was how to express more milk and one in five respondents raised this in their free text response (21%). When asked about lactation problems, 27% reported that they had low milk supply and/or could not express as much milk as they wanted. This focus on milk volumes was seen in all groups, including younger mothers and those from ethnic minority backgrounds.

Respondents were asked to rank seven pre-defined research outcomes identified through the Stage 1 PPI process. Three outcomes were dominant; being able to express more milk (top priority for 32%), being able to breastfeed for longer (top priority for 25%) and feeling that milk supply was more secure (top priority for 19%).

These results gave a strong direction that the primary outcome of the study should be expressed milk yield, and that duration of breastmilk feeding was an important secondary outcome.

Although milk yield was dominant when asked about problems experienced and questions they would like answered, it was not a dominant response when asked what success or improvement

meant in the area of lactation. There was very little consensus on this issue and no single type of response was given by more than 10% of respondents. Table 4.2 shows the diversity of responses.

**Table 4.2: Examples of the diverse range of answers to questions about what 'success' means to parents in relation to breastmilk feeding in prematurity**

| Answers relating to the nature of the breastmilk feeding experience       | Answers related to the duration of breastmilk feeding                                                                                    |
|---------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Being able to express more milk                                           | For longer than actually experienced                                                                                                     |
| Minimising the effect of expressing on time spent with the baby           | Until complementary feeds ("solids") were introduced                                                                                     |
| Providing exclusive maternal milk                                         | Until discharge from hospital                                                                                                            |
| Any direct breastfeeding (getting the baby to latch to the breast at all) | Until the baby was perceived as less vulnerable to infection (for example after winter or when the baby had reached a particular weight) |
| Exclusive direct breastfeeding                                            | Six months from birth                                                                                                                    |
| The mother being happier or more relaxed in relation to lactation         | A year from birth                                                                                                                        |
| Feeling well supported                                                    | Until the baby was a toddler                                                                                                             |
| Improving the baby's weight gain                                          | Until the baby or child decided they wanted to stop breastfeeding                                                                        |
| Feeling listened to and trusted by staff                                  |                                                                                                                                          |

*This table has been previously published<sup>2</sup>*

The stress experienced in relation to lactation was evident from the language used by respondents. Frequently used descriptive words were "struggle" (used 87 times), "problem" (81 times), "difficult" (72 times), "stress" (60 times), "hard" (50 times) and "pressure" (24 times). Descriptions of stress and impact on mental health often revolved around milk volumes and milk supply, and echo the themes identified in Chapter 2:

*"Constantly anxious about expressing enough milk"*

*"[pumping] added to my post-natal trauma and depression. I will never forget how much of a failure . . . I felt."*

*"I was heartbroken that I couldn't do the one thing I thought I should have been able to do!"*

The wide variety of issues raised, including mental health, expressing efficiency and duration of breastfeeding, informed the choice of secondary outcomes. As anxiety was the overwhelming emotion expressed in relation to expressing milk it was a clear choice for psychometric assessment. It was important to many families to breastmilk feed beyond discharge, whereas discharge is often used as a surrogate marker of breastmilk feeding success due to the relative ease of measurement. However, there was no agreement on a single definition of successful breastfeeding for preterm

infants; decision making around duration encompassed not only limitations experienced (such as milk supply or growth pattern), but also parental perceptions of the baby's medical status and fragility, previous plans and the conflicts experienced between lactation plans and mental health. Therefore, the research design incorporated established public health goals (any breastmilk and exclusive breastmilk) at time points that were logistically or physiologically relevant (36 weeks' PMA, two and four months' corrected age). It was also decided to ask participants for their breastmilk feeding goals at baseline to inform subgroup analysis.

#### 4.3.2.3 *Influence - addressing anxiety over milk yield in study design*

A third of respondents (29%) reported at least one concern about the idea of a research study looking at expressing and breastfeeding in preterm infants. The predominant concern was that many mothers have feelings of guilt, judgement, inadequacy and anxiety related to lactation and that being in a study where volumes of expressed milk are monitored and questions asked about breastfeeding and mental health outcomes could exacerbate this, increasing a feeling of pressure or trauma.

Respondents made suggestions to mitigate these risks, which included:

- Communicate sensitively, respectfully and tactfully
- Ensure that families do not feel pressured over breastmilk outcomes but give gentle encouragement, reassurance, support and empowerment
- Be explicit that many mothers have difficulty expressing and breastfeeding and this is 'ok'
- Minimise face to face questions as this could impose more pressure and guilt than filling in details electronically
- Ensure mothers feel that their individual voices are heard

These responses were used to draft the participant facing documents, using appropriate language to reduce any feeling of pressure and ensure that participants were aware that some people can find it hard to express milk. They also supported the decision to predominantly use a more impersonal electronic interface rather than face-to-face data collection.



#### 4.3.2.4 Influence – minimising burden for participants

Some respondents reported a concern that the research study would be an extra burden to participants, taking time away from their baby and intruding into their lives at a stressful time. They recommended making the process as easy as possible to minimise this burden. As noted in Chapter 2, expressing milk was clearly a considerable burden that was in direct competition with maternal needs, so adding an extra component of yield measurement was a concern.

*“... often a choice between sleeping and expressing which leads to horrendous guilt when choosing the former and risking health to prioritise the latter”*

*“Expressing was just difficult due to the time spent between hospital/home/school run/eating, sleeping and showering!”*

In response to these concerns, milk yield monitoring was minimised to three or four time points (depending on gestation at birth), rather than a daily log as used in some studies. Study design was built around electronic data entry, with participants receiving a personalised website link to enter data, in an attempt to make the process as quick and easy as possible.

A tension exists between the desire for high accuracy assessment of milk yield, using milk weight, and the burden on participants, where reading the volume of milk from the container would impose less burden than using a weighing scale. A decision was made to prioritise the use of weights for accuracy while also minimising the number of timepoints that participants are asked to do this. However, it remained clear through the trial that weighing milk was difficult for some participants.

Another area of tension between scientific priorities and the burden on parents was the timing of recruitment. PPI contributors raised concerns that recruiting in the first days after preterm birth could cause distress. However, there are physiological reasons to suspect that interventions to improve milk supply need to occur as early as possible after birth<sup>91,134,222</sup>. Many neonatal trials recruit participants in the first days after birth<sup>223</sup> and some in the first hours after birth where this is required due to a need for urgent action<sup>224</sup>. A decision was made to allow recruitment up to day four after birth to balance these priorities.

#### 4.3.2.5 Influence – additional data analysis

Contributors had many questions about milk expression, summarised in Table 4.3 in order of frequency raised. These questions influenced the choice of outcomes for the exploratory work described in Chapter 7 on expression patterns, and reinforced the importance of assessing parental enjoyment of the relaxation intervention within the randomised trial.

**Table 4.3: Questions for future research on breastmilk expression, from PPI contributors, in order of frequency mentioned**

|                                                                                                                                                                                                      |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| How to increase or maintain milk yield (n = 87). This included mechanical methods (n = 23), food, drink, medication, herbal remedies (n = 17) and methods focusing on connection to the baby (n = 8) |
| How frequently to express (n = 50)                                                                                                                                                                   |
| How long to express for at each session/optimal pattern of expressing (n = 30)                                                                                                                       |
| How best to relax/feel comfortable or happy while expressing milk (n = 25)                                                                                                                           |
| How much milk should be targeted/expected (n = 23)                                                                                                                                                   |
| How best to store breastmilk (n = 11)                                                                                                                                                                |
| The impact of delayed first expression/best timing (n = 10)                                                                                                                                          |
| How to minimise and manage over-supply (n = 9)                                                                                                                                                       |
| Personalised likelihood of being able to express good volumes of milk (n = 9)                                                                                                                        |
| The need for and impact of sterilisation processes for pump equipment (n = 7)                                                                                                                        |
| How to reduce the occurrence of blocked milk ducts and mastitis (n = 4)                                                                                                                              |
| Personalised expectations for the time for milk to 'come in' ('lactogenesis II' or 'secretory activation') (n = 4)                                                                                   |
| The impact of antenatal expressing (n = 3)                                                                                                                                                           |

*This table has been previously published<sup>2</sup>*

Of particular note for the exploratory analysis were the following statements and questions from parents:

*"I think the reality of how difficult expressing every 3 hours plus looking after baby needs to be highlighted. It's almost impossible"*

*"How often should I be expressing? How long each time should I be expressing for?"*

*"Why the pressure to express so frequently and why no recognition of how impossible this is in reality?"*

*"How often to express is actually okay - 10 times a day seemed impossible"*

*"Do you really have to wake up every 3 hours at night as it would put people off"*

*"...what is the optimal frequency of feeding, how often to express and what time of day is best?"*

*"Realistic frequency of expressing as 8-12 times is not realistic when your baby isn't there"*

#### 4.3.2.6 *Influence - dissemination plans*

The questionnaire asked for details about where parents found information about expressing for, and breastfeeding, their preterm baby. Multiple sources of information were reported, both formal and informal. In response to predefined options, the majority (87%) of respondents said that they received information from neonatal unit staff and 40% used printed information found in the neonatal unit. In contrast, only 36% received information from maternity staff, such as a midwife, and 19% used social media for this purpose.

Free text responses listed a wide variety of social media channels and websites used for information (more than 50). Respondents used Instagram, Twitter, Facebook, Pinterest, YouTube and apps as well as websites as sources of information.

A dissemination plan was made using these findings. This focuses predominantly on dissemination to clinical staff, particularly neonatal nurses and neonatal infant feeding leads as the key professionals transmitting information to families. Further materials are being prepared to target families directly via neonatal unit printed information and social media.

#### 4.3.2.7 *Influence - future research topics*

The distinct area of how to successfully transition from expressing (with gavage feeding, where milk is put directly into the stomach via a tube) to direct breastfeeding was frequently raised. Specific questions for future research are listed in Table 4.4, in order of frequency raised by respondents. These areas could not be covered within this programme of research but are important priorities for future research.

**Table 4.4: Questions for future research on direct breastfeeding in prematurity, from PPI contributors; in order of frequency raised**

|                                                                                                                                                     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------|
| How to help preterm babies attach to the breast (n = 50)                                                                                            |
| How to make decisions on the need for gavage supplements when transition to direct breastfeeding has started (n = 19)                               |
| When to try direct breastfeeding for the first time (n = 19)                                                                                        |
| How to transition the breasts from expressing to direct breastfeeding (n = 19)                                                                      |
| The effect of “technology” (for example nipple shields, bottles, pacifiers) on the transition to direct breastfeeding (n = 17)                      |
| How best to support mothers and partners’ lactation, physical and emotional needs (n = 12)                                                          |
| Personalised likelihood of success at transitioning to any/exclusive direct breastfeeding, and how long the transition is expected to take (n = 10) |
| What normal behaviour is for a breastfed preterm baby; for example feeding frequency, night waking, crying (n = 8)                                  |

*This table has been previously published<sup>2</sup>*

### **4.3.3 Stage 3 – Charity collaborator**

#### **4.3.3.1 Structure**

UK national charity Bliss supported the research project from an early stage. One staff member collaborated on the creation of the funding application, trial protocol and trial documents and supported dissemination of the online questionnaire to parents. A second staff member sat on the Trial Steering Committee and has given input into the dissemination planning. Bliss receives funding for their time.

#### **4.3.3.2 Influence**

Bliss advocated for prioritising the mental health and physical needs of parents throughout recruitment and participation in the study, for example, defining an appropriate response to high scores on mental health questionnaires. Bliss influenced the language style of participant facing material to increase accessibility. Bliss also facilitated the very high response rate to the online questionnaire described in Stage 2, through social media dissemination.

#### **4.3.4 Stage 4 – Online panels**

##### *4.3.4.1 Structure*

A subset of 49 questionnaire respondents who had given consent for further contact and whose infants were under 32 weeks' gestation at birth were invited to help review trial documents. This included all respondents who were under 25 years old at the birth of their infant or who were from an ethnic minority background. A formal role description was provided with the offer of associated payment in line with INVOLVE guidance<sup>221,225</sup>.

Two panels were set up using videoconferencing software. Both were scheduled in an evening slot timed with the hope that contributors' children might be asleep. Panellists were reassured that they could come and go according to their children's needs. They were asked in advance whether they had any special requirements to maximise participation and sent documents to read in preparation.

Eleven mothers wanted to be involved in the online panels (22% of those invited) and were sent trial documents. Four contributors attended one of two online panels and two further mothers sent written feedback instead (12% of those invited). Two were from Black and other minority ethnic backgrounds and one was under 25 years old. There was a range of gestational age experiences and one mother had experienced neonatal bereavement.

Initial activities were targeted at putting participants at ease, sharing their background and setting ground rules. Each document was discussed in turn and then pre-specified targeted questions were asked on particular terminology and issues that the researcher had experienced while writing the trial protocol. Finally, there was an opportunity for contributors to comment on the trial design and raise other points.

It was also planned to discuss the documents in person one to one with parents currently in a neonatal unit to focus on demographics that were under-represented in the online questionnaire; for example mothers from non-European countries, teenage mothers and mothers from lower

socioeconomic backgrounds. Unfortunately, this was not possible because of the SARS-CoV-2 pandemic restricting non-clinical presence in the hospital at that time.

#### *4.3.4.2 Influence – language and focus*

Panellists' feedback led to changes in language used and new documents being created, for example, a short introductory leaflet that would be less overwhelming than the full Participant Information Sheet. Free-text questions were added to the participant Case Report Forms to make them feel more accessible and encourage a relationship where the trial participants would feel heard as individuals. Resources were added to the end of each Case Report Form directing people to sources of support related to lactation, mental health and having a preterm baby.

#### *4.3.4.3 Influence – choice of psychometric instrument*

Panelists were shown a range of psychometric instruments covering both depression and post-traumatic stress (PTS) reactions. The anxiety instrument had already been chosen (the Spielberger State Trait Anxiety Index) and because a limited number of secondary outcomes could be included within the constraints of the sample size, one further instrument needed to be chosen. There was a clear preference for assessing PTS reactions and for the Post-Traumatic Check List for DSM-5 (instead of the Impact of Event Scale – IES) due to the tone of the language used in the questions. This preference for PTS assessment over depression was because contributors felt that PTS reactions are common after very preterm birth, poorly identified and are a priority for research.

#### *4.3.4.4 Influence – additional training needs*

One panellist focused on the need for specific bereavement training for research staff because of the risk of participants experiencing the loss of their baby, or one of their babies for mothers of multiples. All research staff were therefore recommended to undertake bereavement training.

#### 4.3.5 Stage 5 – RCT intervention modification exercise

##### 4.3.5.1 Structure

All respondents who had given consent for further contact were invited to help choose and modify the relaxation recording to be used as the RCT intervention (approximately 250 invitations). The invitation emphasised that people from minority backgrounds were particularly welcomed. A formal role description was provided with an offer of associated payment in line with INVOLVE guidance<sup>221,225</sup>.

Sixty-two people responded to the invitation (25%). Twelve were selected to include a range of gestational ages at birth and to include people from diverse backgrounds. Numbers were limited due to the PPI payment budget. Maternal age ranged from 25 to 48 years. One third of the group came from a minority ethnic background and two spoke English as a second language.

Two draft audio files were sent to contributors, both of which had been used in previous RCTs<sup>172,202,203</sup>. Initial modifications had been made to these recordings using themes raised in the Stage 2 questionnaire. Contributors were asked for detailed feedback, both open ended and in response to specific questions, and to choose their preferred recording. Once final modifications were complete, all contributors were sent feedback on the impact of their involvement.

##### 4.3.5.2 Influence – intervention choice and modification

One recording was clearly preferred by the majority of contributors. This recording script was then further modified based on three key areas identified; reducing a feeling of pressure and judgement on having a low milk supply; language and imagery appropriate for sick, immature babies; and language and imagery appropriate for women with traumatic pregnancy and birth experiences.

Examples of comments on these themes are:

*“...there are some things to consider such as [reference to] uterus and internal organs - a mother may have had a c section and possibly even a hysterectomy during delivery”*

*“The skin to skin imagery was lovely. I would have found it quite emotional to hear “you can grow to trust your body” especially after feeling that my body failed me hence my baby being premature. But this is not necessary a negative thing”*

*"I wonder if being asked to visualise holding your baby when in reality you probably can't would be upsetting to hear. I'm also not keen on some of the language around giving the gift of milk to your baby, etc - I think this would increase the feeling of pressure, especially if the mother is struggling to produce"*

Appendix B.2 shows the recording script modifications in full using "tracked changes".

#### **4.3.6 Stage 6 – Trial Steering Committee**

##### *4.3.6.1 Structure*

One staff member from Bliss and one parent who had been part of the PPI online panels (stage 4) were invited to sit on the Trial Steering Committee, which met three times before, during and after the trial was complete.

##### *4.3.6.2 Influence*

The Steering Committee PPI representatives recommended actions to increase data completeness and advised on different potential incentives that could be offered to increase positivity about the trial. For example, the PPI representatives helped to explore whether to send participants a voucher or a gift, which was an amendment put in place after several months to attempt to raise data completeness. They reviewed the format of an option to upload photos of paper Case Report Forms that was introduced as an amendment to increase return of these forms for those who didn't want to use the electronic format and found the existing Freepost option off-putting.

#### **4.3.7 Stage 7 – Dissemination**

##### *4.3.7.1 Structure*

Three months after the completion of trial follow up, 27 invitations were sent to trial participants to provide input into dissemination of trial results. Invitees were selected in relation to their demographic characteristics and their expressed milk yield to ensure diversity of background and outcomes. Five participants accepted the invitation (19% of those invited). Individual and group meetings were set up to informally present and discuss the results, either in person, virtually or by



telephone according to participant preference. Draft dissemination materials were also emailed and/or posted to participants for feedback.

#### *4.3.7.2 Influence*

Participants recommended to share all trial results and lots of qualitative comments in order to fully and honestly 'tell the story'. They also wanted to hear about how the study results fitted into the general literature. Participants recommended the use of visual cues to explain the magnitude of results, such as photos of expressing containers containing milk.

When discussing the exploratory trial results, which relate to clinical recommendations about how to express milk, participants recommended including lots of reassurance and acknowledgement about feelings of guilt and inadequacy relating to whether or not they could follow recommendations, and practical advice to support putting the evidence into practice.

Not all of these recommendations could be put into practice, as there was tension between including as much content as possible and keeping language simple and documents at an accessible length. One way of navigating this tension was to create a shorter and simpler 'easy read' document and a longer version including more detail and qualitative content. Social media infographics were also designed to increase accessibility and diversity of messaging. These dissemination documents are provided in appendices when the relevant results are presented in Chapter 6 and 7.

### **4.3.8 Summary of influence and impact**

Key impacts of PPI for this trial were in defining the study outcomes, minimising participant burden/anxiety through trial design, modifying the intervention and informing dissemination efforts. The key impact for future work is providing other research questions identified by parents. Table 4.5 describes the nature of the influence of PPI on the research project using the Public and Patient Engagement Evaluation Tool (PPEET) for inspiration.

**Table 4.5: Summary of PPI influences**

| Type of influence                                           | Amount of influence | PPI stage involved                                                                                       | Comment                                                                                                                                      |
|-------------------------------------------------------------|---------------------|----------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| Choice of population and intervention category              | Moderate            | Informal PPI (stage 1)                                                                                   | Set before formal PPI started                                                                                                                |
| Choice of trial outcome measures                            | Large               | Informal PPI (stage 1)<br>Online Questionnaire (stage 2)<br>Charity Collaborator (stage 3)               | Formal PPI informed the choice of specific outcomes from a range of possibilities and their definitions (for example, timepoint assessed)    |
| Areas for exploratory analysis                              | Moderate            | Informal PPI (stage 1)<br>Online Questionnaire (stage 2)                                                 | Formal PPI confirmed areas of focus and provided more detail on questions of interest                                                        |
| Trial design – minimising adding to anxiety over milk yield | Large               | Online Questionnaire (stage 2)<br>Charity Collaborator (stage 3)<br>Online Panels (stage 4)              | Amendments to processes and documents                                                                                                        |
| Trial design – minimising burden for participants           | Large               | Online Questionnaire (stage 2)<br>Charity Collaborator (stage 3)<br>Online Panels (stage 4)              | Amendments to processes and documents. Some areas of tension decided by researcher                                                           |
| Trial modifications in response to challenges               | Small               | Trial Steering Committee (stage 6)                                                                       | Consultation. Areas of disagreement decided by researcher                                                                                    |
| Intervention content                                        | Very Large          | Informal PPI (stage 1)<br>Online Questionnaire (stage 2)<br>Intervention Modification Exercise (stage 5) | Iterative changes with detailed PPI input. Some disagreement among participants – final decision by researcher                               |
| Dissemination plan                                          | Very Large          | Online Questionnaire (stage 2)                                                                           | Detailed list of vehicles of communication for dissemination provided by PPI, along with assessment of most significant channels             |
| Dissemination content                                       | Large               | Dissemination (stage 7)                                                                                  | Open discussion to set the framework of document design. Iterative changes to dissemination material. Areas of tension decided by researcher |
| Future research topics                                      | Large               | Informal PPI (stage 1)<br>Online Questionnaire (stage 2)                                                 | Broad range of questions posed                                                                                                               |

*The Public and Patient Engagement Evaluation Tool is licensed under a Creative Commons Attribution-Non Commercial-Share Alike 4.0 International License. ©2018, Julia Abelson and the PPEET Research-Practice Collaborative. McMaster University. All rights reserved.*

*This table has been previously published<sup>2</sup>*

## 4.4 Discussion

### 4.4.1 Strengths and Limitations

Parent involvement had a far-reaching influence on this programme of work during the planning and design phase, which reinforces the importance of PPI at the earliest stages of the research cycle<sup>213</sup>. The online questionnaire format elicited an unexpectedly deep and broad pool of insights to influence trial design and the number of people involved was much larger than other published PPI reports<sup>214,226,227</sup>. However, a known weakness of using social media to recruit participants<sup>228</sup> is that it is less effective at including marginalised voices, for example, families from ethnic minority backgrounds and communities with lower socioeconomic status. This is a frequent theme in PPI, where the people who are easiest to engage are often the most privileged<sup>219,220</sup>. Thus 95% of online questionnaire respondents were White, whereas only 71% of people giving birth in the UK are White<sup>229</sup>, and those from ethnic minority backgrounds have higher rates of preterm birth<sup>230</sup>.

However, the very large response to the online questionnaire meant that even though more rarely reached groups were under-represented proportionally, their voices are still present. The involvement of more than 50 young mothers and 30 mothers from ethnic minority backgrounds is a very positive achievement within a PPI setting.

Asking about contributor demographics in the online questionnaire meant that minority voices could be preferentially studied and invited for further steps of PPI work. This meant that a third of the panellists and recording exercise contributors were from ethnic minority backgrounds and several were under 25 years old. Despite the strength of this over-sampling approach, it was limited by the data gathered at the questionnaire stage. For example, respondents were not asked about income or education level, or protected characteristics such as disability and sexual orientation, so there are likely areas of under-representation that were not identified or over-sampled. In addition, intersectional communities are less likely to be represented, for example, people from ethnic minority groups who also have low incomes. The decision to limit the number of demographic

questions was due to the potential for participants to find certain questions stigmatising and invasive in the setting of an online questionnaire.

At the dissemination stage there was a much more diverse population to draw from because trial participants were invited to contribute (trial participant demographics are presented in Chapter 6; this approach was discussed with and supported by the sponsor). However it was notable that those who responded to the invitation were predominantly highly educated, showing that performing diverse PPI can be more challenging than diverse trial recruitment. This may be because the PPI invitation was sent by email, which is more impersonal than face to face contact. Other reasons might relate to literacy levels, consideration of potential personal benefit or expectations about what PPI would involve. The one participant without a degree level education required more support to be involved, with individual explanations over the phone and material printed and posted rather than emailed.

There were many aspects of best practice within the PPI process. For example, contributors were adequately compensated (according to INVOLVE guidance<sup>221,225</sup>) to reduce barriers to involvement and to show the value of their 'symbolic capital'<sup>231</sup> as experienced parents with a deep understanding of the trial context. Role descriptions were provided to create a shared understanding of expectations and live interaction was planned to maximise inclusion of this particular population with young children. However, around two thirds of those who wanted to participate in online panels did not end up attending which shows the difficulty of providing accessible arrangements for parents of young children. A range of available times or individual consultations may have been preferable and this was put into practice for the dissemination stage, with everyone who wanted to contribute being able to do so in a way that suited their own circumstances.

A limitation of the PPI design for this project was that the scope and areas of influence of the partnership were predominantly directed and designed by the researcher. There was an unequal balance of power with PPI contributors unable to ensure that their concerns were acted upon in

any areas of tension or disagreement. Therefore, the level of participation was predominantly 'consultation' with some elements of 'partnership' rather than higher levels of 'delegated power' or 'citizen control'<sup>232</sup>.

#### **4.4.2 Putting findings into context**

Some actions resulting from PPI in this trial are similar to the conclusions from other neonatal and paediatric PPI conducted while planning RCTs<sup>226,227</sup>. For example, providing participant information in short formats to cater for parents with different information needs, a focus on compassionate language when describing low volumes of maternal milk<sup>226</sup> and giving participants the opportunity to describe their experiences more freely, rather than being constrained to validated questionnaires and numerical responses<sup>227</sup>.

However, the broad, foundational questions asked in the online questionnaire, and the very large number of responses at an early stage, meant that this PPI process has resulted in a deeper and wider set of transferrable insights than most reports<sup>214,226,227</sup>. Thus, in addition to practical changes to trial design, a wide range of future research topics that parents want answers to have been presented, which can be a springboard for the research community.

#### **4.4.3 What could be done differently**

The PPI process relied heavily on digital literacy and access because of the limitations imposed by the SARS-CoV-2 pandemic. Although this allowed a broader reach and, in some cases may have increased accessibility for parents of young children, additional strategies are needed to include those in digital poverty and with communication barriers<sup>219</sup>.

Future PPI should be structured to increase the power of PPI participants in the research process. Even without structural changes to integrate shared decision making, contributors can be given more opportunities to challenge the power dynamic by providing anonymous feedback on how the PPI was conducted<sup>233</sup>, for example using the PPEET questionnaires<sup>234</sup>.

Some of the limitations of this process were the result of PPI taking place without initial funded resource, and some were due to the inexperience of the researcher. Both are acknowledged barriers to high quality PPI within doctoral research<sup>214</sup>, although seen at all levels<sup>213</sup>. University Departmental PPI networks and support systems could help overcome resource pinch points and provision of expertise.

## **4.5 Summary**

This chapter has described the iterative process of bringing the voices of parents into this programme of research at every stage in the research lifecycle. The broad influence of the PPI process has been discussed, as well as limitations and outcomes that relate to future research. The next chapter will describe in full the methods of the randomised research design that this PPI process fed into.

# Chapter 5: RCT Methods

---

## 5.1 Chapter Overview

Chapter 3 outlined the evidence supporting the potential effect of relaxation interventions on lactation and Chapter 4 described the process of parental involvement in designing a new randomised controlled trial in this area. This chapter will describe the resulting protocol for the experimental work performed. Much of the material has been published as a summary trial protocol in *Trials*<sup>1</sup>. I am the lead author of this paper. The co-authors are three doctoral supervisors and three Clinical Trials Unit colleagues in the National Perinatal Epidemiology Unit. I led the planning of the research methods and drafted the protocol. The co-authors supported the research design and gave feedback on the protocol. All co-authors have given permission to use relevant material from the published paper in this thesis. The published paper is provided for reference as Appendix C.

## 5.2 Methods and objective

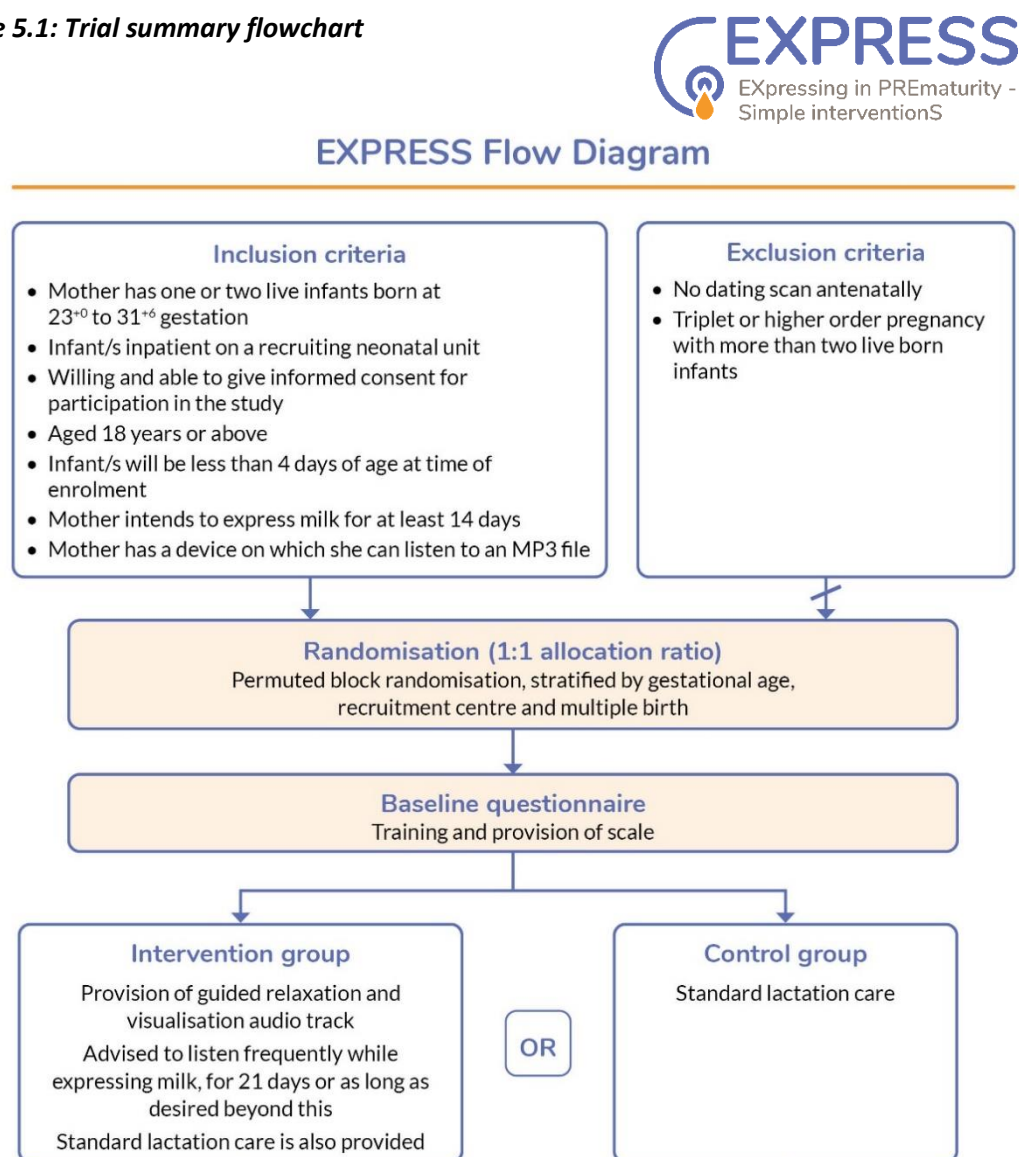
This was a multi-centre, unmasked, randomised, controlled, parallel group trial designed to determine if provision of a self-directed guided relaxation and lactation-specific visualisation to mothers of infants born from 23+0 weeks' PMA to 31+6 weeks' PMA improves lactation and mental health outcomes. Both groups received standard clinical support for lactation provided by their neonatal unit staff. The intervention was provision of a 12-minute long modified audio recording that participants were requested to listen to frequently while expressing breastmilk. The primary objective is outlined in Table 5.1. As a real-world study, the intervention was the provision of the recording, with the knowledge that not everyone who is given or recommended a behavioural intervention in clinical practice will adhere, and that adherence level will vary by individual.

**Table 5.1: Primary Objective in PICO format**

|                          |                                                                                                                                                            |
|--------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Population</b>        | Mothers of one or two infants born between 23+0 and 31+6 weeks' post-menstrual age                                                                         |
| <b>Intervention</b>      | Provision of a specific guided relaxation and lactation-specific visualisation recording with instruction to listen frequently while expressing breastmilk |
| <b>Control</b>           | Standard care, including standard lactation support provided by neonatal unit staff                                                                        |
| <b>Outcome - primary</b> | Expressed milk yield in 24 hours (highest value recorded on any of day 4, day 14 or day 21 after birth)                                                    |

Data was submitted directly by participants into Case Report Forms (CRFs) and through participant responses to short message service (SMS) messages. Research staff entered baseline and secondary outcome data from the participant and infant medical notes. The trial aimed to recruit a total of 132 participants. The trial design is summarised in Figure 5.1.

**Figure 5.1: Trial summary flowchart**



*This figure has been previously published<sup>1</sup>*

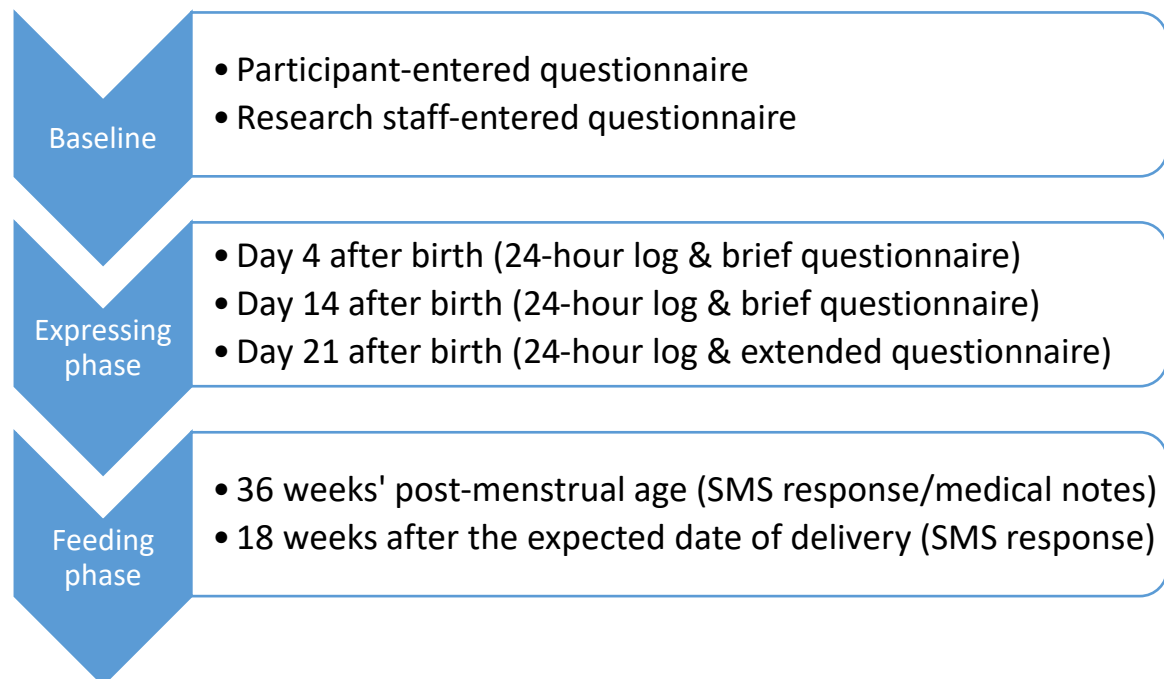


Data was collected at six timepoints, described in Figure 5.2. At baseline, the participant completed a CRF questionnaire about themselves, their previous breastmilk feeding experience and feeding intentions. Research staff completed a trial entry CRF with information about the birth and infant's medical status at randomisation.

In the expressing phase of data collection, the participant completed a CRF questionnaire and 24-hour log of expressed milk at three timepoints; day four after the infant's birth (the day of birth is termed day zero), day 14 and day 21 after the infant's birth.

In the feeding phase of data collection, the participant responded to an SMS message asking about the infant's feeding status, at two timepoints. These were 36 weeks' post-menstrual age (PMA; four weeks before the expected date of delivery, the EDD, which defines 40 weeks of gestation), and 18 weeks after the EDD. Their SMS response automatically completed a CRF for each timepoint. If no SMS were received, infant feeding status could be extracted from the medical notes (at 36 weeks' PMA only).

**Figure 5.2: Timepoints for trial data collection**



*This figure has been previously published<sup>1</sup>*

For reasons of efficiency within the available time for a doctoral thesis, the recruitment period was divided into two sections. The majority of recruitment (10 months) took place in the ‘complete follow-up’ period where all participants randomised were followed up at 36 weeks’ PMA and 18 weeks’ corrected age (receiving both SMS messages). The final 22 weeks of recruitment formed the ‘abbreviated follow-up’ period where all participants randomised were followed up at 36 weeks’ PMA only (receiving only the first SMS message). The schedule of trial procedures is provided in Table 5.2.

**Table 5.2: Schedule of trial procedures**

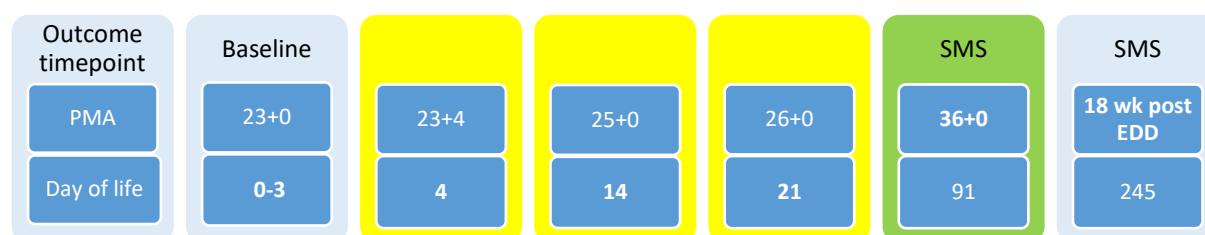
| Procedures                                        | Day of birth to midnight on day 3 | Day 4, 14 | Day 4 to 7 | Day 21 | 36 weeks’ PMA & 18 weeks after EDD* |
|---------------------------------------------------|-----------------------------------|-----------|------------|--------|-------------------------------------|
| Informed consent                                  | ✓                                 |           |            |        |                                     |
| Eligibility assessment                            | ✓                                 |           |            |        |                                     |
| Randomisation                                     | ✓                                 |           |            |        |                                     |
| Baseline Questionnaire                            | ✓                                 |           |            |        |                                     |
| Maternal training and assessment on use of scales | ✓                                 |           |            |        |                                     |
| Check-in and verify accurate use of scales        |                                   |           | ✓          |        |                                     |
| Questionnaire & 24-hour expressing log            |                                   | ✓         |            | ✓      |                                     |
| Maternal mental health questionnaires             |                                   |           |            | ✓      |                                     |
| Text message response                             |                                   |           |            |        | ✓                                   |

*\*In the abbreviated follow up period the trial completes at 36 weeks’ PMA. PMA = post-menstrual age. EDD = expected date of delivery*

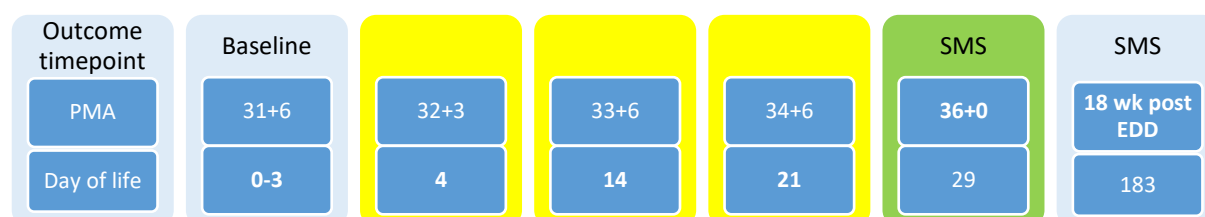
The time from recruitment to the end of the expressing phase of the study was three weeks. The time from recruitment to the end of the feeding phase of the study varied with the gestation of the infant and the follow up period. The longest possible time (for a participant recruited in the ‘complete follow-up’ period with an infant born at 23 weeks) was 35 weeks. The shortest possible time (for a participant recruited in the ‘abbreviated follow-up’ period with an infant born at 31 weeks and six days) was four weeks. Figure 5.3 shows sample timelines for the most and least preterm infants.

**Figure 5.3: Sample timeline for most and least preterm infant eligible**

#### **Most Preterm**



#### **Least Preterm**



PMA = post-menstrual age. Timepoints considered for the primary outcome assessment are highlighted in yellow. The end of the study for those recruited in the 'abbreviated follow-up' period is highlighted in green. This figure has been previously published<sup>1</sup>

### **5.2.1 Setting & participants**

The trial recruited participants within three National Healthcare Service Trusts in northern and southern England (Manchester, Oxford and London), covering four individual neonatal units (three tertiary level intensive care units and one local neonatal unit). All sites had at least one dedicated infant feeding support professional and counselling or psychological support available. All sites had hospital-grade pumps available for use in the hospital and a loan scheme for free use at home. Breastmilk feeding rates at discharge were variable, with one site below the regional and national averages and the others above average<sup>235</sup>. Two Trusts (three neonatal units) had level 3 UNICEF UK Baby Friendly Initiative accreditation and one has not yet started the accreditation process. All sites are urban and serve a range of communities, including diversity of ethnic background and social deprivation.

Participants were mothers of infants born between 23+0 and 31+6 weeks of gestation. Full inclusion and exclusion criteria are provided in Figure 5.1. Participants with more than two live born infants were excluded and participants had to have an intention to express milk for at least two weeks after birth to join the trial. Participants could be screened by research nurses and trained clinicians during

pregnancy and after birth. Participants had to provide written, informed consent (either on paper or electronically) and were provided with written information sheets. Randomisation could only take place between birth and midnight on day three after birth.

### **5.2.2 Description of Intervention**

The intervention was the provision of a specific recording to the participant as an MP3 file with a request to listen frequently during expression of milk, on their own device, for at least three weeks. The intervention recording lasted approximately 12 minutes and consisted of a guided relaxation and lactation-specific visualisation. This was a modified version of an existing soundtrack used for previous studies<sup>172,195,197,203</sup>, with a license from the original author for modification, use and distribution. The visualisation includes descriptions of pleasant surroundings, milk flowing in the breasts, and skin-to-skin contact with the infant. The script is provided as Appendix B.2 and the reasons for and process of modification was described in Chapter 4. The modified version was made freely available after the trial completed ([www.npeu.ox.ac.uk/express/download-relaxation](http://www.npeu.ox.ac.uk/express/download-relaxation)).

Both intervention and control groups received general lactation care, including routine advice from neonatal unit staff and standardised printed information on milk expression complying with UNICEF UK BFI recommendations<sup>24</sup>.

### **5.2.3 Randomisation**

Participants were randomised with a 1:1 allocation using stratified permuted block randomisation by a secure web-based randomisation program hosted by the National Perinatal Epidemiology Unit Clinical Trials Unit (NPEU CTU). Stratification was based on recruitment neonatal unit, gestational age at birth (23+0 to 27+6 weeks versus 28+0 to 31+6 weeks) and multiple birth (one versus two infants alive at time of randomisation). Study identification numbers were not consecutive, to reduce the possibility of research staff monitoring the sequence of allocation.

Research staff and the participant were aware of the allocation after randomisation, as the study was unmasked. However, efforts were made to maintain blinding of data outputs to the majority

of staff (including myself) until the data were finalised and locked. For example, pre-specified data monitoring extracts were performed by a member of the CTU programming team and interim analysis for the data monitoring committee was performed by independent statisticians outside the NPEU CTU. In preparing statistical analysis programs during the period of recruitment and follow up, I used dummy data. Blinded and unblinded data downloads were kept in separate locations and I did not gain access to the unblinded location until after the data were locked.

#### 5.2.4 Primary outcome

The primary outcome was defined as the highest 24-hour expressed milk weight recorded on any of day 4, day 14 or day 21 (in grams). If the participant declared that they were no longer expressing, the milk weight for that day was accorded a value of zero. Table 5.3 shows various scenarios of what this means in practice. The reason for this definition of the primary outcome, instead of using 24-hour expressed milk weight at a specific timepoint such as day 21, was that some more mature infants may start to directly breastfeed by day 21 and this is likely to decrease expressed milk yield. In addition, some parents may choose to stop expressing before day 21 but increasing their milk yield prior to this would still be an important outcome to reduce infant morbidity. Finally, the physiology of lactation studied in term infants<sup>131</sup> suggests that once milk supply is established it is robust to supply and demand; therefore achieving a higher milk yield at day 14 than day 21 may suggest that the parent will be able to increase their yield as needed with more frequent expressing or breastfeeding in the future.

**Table 5.3: Examples of primary outcome calculation**

|                   | Day 4 | Day 14  | Day 21                  | Primary Outcome |
|-------------------|-------|---------|-------------------------|-----------------|
| <b>Scenario 1</b> | 300g  | 500g    | 750g                    | <b>750g</b>     |
| <b>Scenario 2</b> | 100g  | 450g    | 400g                    | <b>450g</b>     |
| <b>Scenario 3</b> | 50g   | missing | missing                 | <b>50g</b>      |
| <b>Scenario 4</b> | 50g   | 150g    | Stopped expressing (0g) | <b>150g</b>     |

*This table has been previously published<sup>1</sup>*

### 5.2.5 Secondary outcomes

The following were the secondary outcomes:

- Proportion of participants expressing at least 750g of milk in 24 hours on any of day 4, 14 or 21
- Rate of milk expression (in grams per minute) at day 21
- Average Spielberger State Trait Anxiety Index (six item format; STAI-6) score at day 21
- Average Post-traumatic Stress Checklist for DSM-5 (PCL-5) score at day 21
- Proportion of participants feeding their infant/s only breastmilk (no infant formula) at 36 weeks' PMA
- Proportion of participants feeding their infant/s any breastmilk at 36 weeks' PMA
- Proportion of participants feeding their infant/s only breastmilk (no infant formula) at 18 weeks after the estimated date of delivery (four months' corrected age)

For the outcome of any breastmilk at 36 weeks' PMA, data could be taken from parent response to an SMS or from a previous report of lactation cessation. For the outcomes of exclusive and any breastmilk at 36 weeks' PMA, feeding data could also be extracted from the infant medical notes if required.

The STAI is a reliable measure of anxiety<sup>236</sup> and widely used, including in studies after very preterm birth<sup>207,237</sup> and after use of a relaxation intervention in the neonatal intensive care unit<sup>174</sup>. The original STAI is a 20-item questionnaire with four answer options for each question ('not at all', 'somewhat', 'moderately' or 'very much'). The total score range is 20–80, with higher scores signifying more anxiety, <36 generally considered normal<sup>238</sup> and ≥40 signifying clinically significant anxiety<sup>207</sup> (although this cut off varies across the literature<sup>67</sup>). The shortened STAI-6 contains a subset of six questions, giving a score total of 6–24, which are then scaled to 20–80 for comparability<sup>239</sup>. The short form is highly correlated with the 20-item STAI, with internal consistency greater than 0.9<sup>240</sup>. The minimal meaningful difference in STAI score has been suggested as 10<sup>241</sup>.

The PCL-5 is a commonly used<sup>242</sup> publicly available screening assessment for post-traumatic stress disorder (PTSD) aligned to the Diagnostic and Statistical Manual of Mental Disorders-Fifth Edition (DSM-5)<sup>243</sup>. It has been used in the context of birth related trauma<sup>244</sup>. The PCL-5 is very similar to the Impact of Event Scale (IES) and the preceding PCL for DSM-IV that have both been used in the specific context of preterm birth<sup>207,245</sup>.

The PCL-5<sup>243</sup> has 20 items with four answer options for each question (“not at all”, “a little bit”, “moderately”, “quite a bit” and “extremely”). The total score range is 0–80, with higher scores signifying more distress. A cut off of 31–33<sup>246,247</sup> has been proposed as indicative for probable PTSD. A 5–10 point change is likely to be the minimum threshold for a clinically meaningful difference<sup>243</sup>. The PCL-5 has internal consistency (for example, Cronbach’s alpha 0.95) and good construct validity in a variety of settings<sup>246–248</sup>. Of note, a diagnosis of PTSD requires symptoms to continue beyond 28 days after the traumatic event; therefore in this context the PCL-5 measures report of post-traumatic stress reactions rather than PTSD.

In relation to the secondary outcomes measured at 36 weeks’ PMA and four months’ CA, note that no reference is made to whether the infant is having complementary feeds (“solids”) in addition to milk. This is because the timing of introduction of complementary feeds for preterm infants is not recommended by actual age as it is with term infants, but rather personalised to the developmental stage, actual and corrected age of the infant<sup>249</sup>.

#### **5.2.6 Process indicators**

The following were the process indicators:

- Time spent in skin to skin contact with infant/s at day 21 (in hours)
- Number of expressing episodes in 24 hours at day 21
- Total time spent expressing in 24 hours at day 21 (in hours)

The process indicators were chosen as they may indicate a behavioural pathway for any effect seen on the primary outcome.

### **5.2.7 Monitoring adherence**

At day 4, 14 and 21, participants in the intervention group were asked how many times they had listened to the intervention in the previous 24 hours. At day 21 both groups were asked how often they had practised other (non-intervention) forms of relaxation since the birth of their infant/s.

### **5.2.8 Measures to improve data quality and retention**

Research nurses and trained clinicians had close contact with participants during the expressing phase. At baseline, participants were given written information on weighing milk and using the electronic forms; short videos were also available. Research staff contacted participants after the first expressing log to ensure that the weighing record was accurate and to respond to questions. Automated reminder emails and text messages were sent to participants if forms were not filled in. Trial staff checked participant-submitted forms and research staff raised any obvious problems with participants in a timely period. Automated text messages were sent to the intervention group on day 9 and day 17 after birth to remind them to listen to the intervention. Prior to each feeding phase SMS, emails were sent explaining their importance.

### **5.2.9 Withdrawal and change in consent**

Each participant had the right to change their consent at any time and was not contacted for further data submission after this time. In addition, the Investigator could withdraw a participant from the study at any time if the Investigator considered it necessary for any reason including:

- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Death of the mother or the infant/s during the study period

The number of participants withdrawn and changing their consent status was reported by trial arm, with reasons. Participants choosing to stop listening to the intervention recording but continuing follow-up remained in the study. Participants could choose to continue in the study after the death of a single twin.



### 5.2.10 Data collection

At day 4, 14 and 21, participants were asked to record information about each time that they attempted to express milk in a 24-hour period, starting when they woke up for the day. Participants were trained at recruitment to weigh their expressed milk inside its container and to record the weight of the empty container. At analysis the pure milk weight expressed could therefore be calculated and summed over the 24 hour period. Each participant was given a portable digital scale accurate to 0.1g (Kabalo).

Participants could fill in the expressing log and questionnaire on paper or on an electronic form with individual URL, hosted by OpenClinica (a third party application hosted in the UK). Paper forms could be sent by post or photographs could be uploaded securely by participants (the latter option was added half way through the trial by amendment as there were significant postal delays and getting to a post box was noted to be a barrier to submission). Participants received automated notifications the night before and on the morning of the scheduled day, and a reminder the next day if appropriate. The scheduled period for data response was within 48 hours of each request.

Participants could express milk by whatever method they desired. By day four after birth, all sites recommended participants to use a double hospital-grade electric pump.

At 36 weeks' PMA and four months' CA, participants were sent an SMS asking what type of milk their infant/s was drinking; only breastmilk, only infant formula or a mixture of the two. The scheduled period for data response was within 7 days of the SMS.

Basic demographics of the potentially eligible population were extracted from routinely entered clinical data in the BadgerNet platform and were fully anonymised before transfer to the NPEU, as a form of screening log.

No formal safety reporting was required due to the low risk nature of the intervention.

### 5.2.11 Project Management

The trial was run on a day-to-day basis by myself, supervised by the Project Management Group (PMG), which reported to the Trial Steering Committee (TSC). The TSC received recommendations related to the data arising from the trial from the Data Monitoring Committee (DMC). The PMG consisted of myself, the Clinical Trials Unit Director, Head of Operations, Senior Trials Manager, Trial Programmers, Trial Statistician and Trial Administrator. Two DPhil supervisors also attended the PMG, one a clinical neonatologist. The PMG met every three to four weeks. The TSC and DMC consisted of independent clinical and statistical experts, as well as parent and public representatives. The TSC and DMC met three times over the course of the trial. Apart from myself (sitting on the TSC), all members of the TSC and DMC were independent of the CTU and the sponsor. The trial was run according to the Clinical Trials Unit standard operating procedures, including quality assurance monitoring and the potential for audit. The sponsor was the University of Oxford, which has ultimate responsibility for the trial's scientific integrity.

### 5.2.12 Statistics and analysis

The full RCT Statistical Analysis Plan (SAP) can be downloaded from the ISCRTN registration at [www.isrctn.com/ISRCTN16356650](http://www.isrctn.com/ISRCTN16356650). An abridged SAP is provided in Appendix D, with elements that are described in detail in this chapter removed to avoid duplication.

#### 5.2.12.1 *Sample size and power calculation*

In the most relevant previous study of those published at the time of powering the study (the most similar to the proposed population and intervention)<sup>203</sup>, mean expressed milk yield at day 14 of the trial increased from 318ml (standard deviation; SD 309ml) to 862ml (SD 309ml) with the use of a visualisation/relaxation soundtrack. Initial exploratory work suggested that baseline milk yield in the recruiting sites was higher than this and therefore my study was powered to detect an increase in highest 24-hour expressed milk yield from 670g (SD 300g) to 825g (SD 300g), with 80% power and a two-sided significance level of 0.05. This is a 155g absolute mean difference or a 0.5 standardised mean difference.

118 participants were required, so the recruitment target was set at 132 to allow for 10% attrition. Since breastmilk has an average specific gravity of 1.03<sup>250,251</sup>, 670g is estimated as equivalent to 650ml and 825g is estimated as equivalent to 800ml, although much of the literature views volume and mass measurements as interchangeable for breastmilk<sup>38,252–254</sup>.

It was acknowledged from the planning stage that a smaller difference in milk quantity than 155g would have clinical impact in the setting of very preterm birth (as outlined in Chapter 2, each 10ml/kg/day of mother's milk is associated with improved medium and long term outcomes). However the limitations of the number of recruitment centres possible within a doctoral RCT, the time available and the relatively low frequency of very preterm birth meant that powering the study to detect the minimal clinically impactful mean difference was not possible.

#### 5.2.12.2 *Description of statistical methods*

Demographic and clinical data were summarised with counts and percentages for categorical variables, means (with SDs) for normally distributed continuous variables and medians (with interquartile ranges) for other continuous variables.

Statistical analysis of continuous outcomes used linear regression with adjusted and unadjusted mean differences presented, or quantile regression with median differences presented, as appropriate. Binary outcomes were analysed using log binomial regression, or Poisson regression with a robust variance estimator if the model failed to converge. Risk ratios with corresponding 95% confidence intervals were presented.

Analyses were adjusted for the stratification factors (recruiting neonatal unit, gestational age at birth and whether the participant had one or two infants alive at randomisation) where possible. The STAI score at day 21 was adjusted for the STAI score at baseline. The primary outcome was also adjusted for the associated measurement day of the highest milk weight. Both crude and adjusted estimates were presented, but the primary inference was based on the adjusted estimates. Further process indicators, such as number of direct breastfeeds, were summarised by randomised arm, with no comparative statistics presented.

Participants were analysed in the groups to which they were randomly assigned, comparing the outcome of all participants allocated to intervention with all those allocated to the comparator group, regardless of deviation from the protocol or treatment received (the Intention to Treat population). Analysis took place on a superiority basis.

Exploratory subgroup analysis used a statistical test of interaction to examine the effect of the intervention on the primary outcome by gestational age at birth (<28 weeks and ≥28 weeks). Stratified analysis by the other stratification factors and by exclusive breastmilk feeding intention at baseline took place with no formal test for interaction. This was to limit multiple comparisons and due to the low statistical power for subgroup analysis.

An exploration of adherence effect on the primary outcome was conducted, dividing the intervention group into high and low frequency adherence by their reported number of times listening to the recording. An exploration of perceived relaxation effect on primary outcome was conducted, dividing the intervention group into those who reported the intervention to be relaxing and those who did not (via Likert scale). Summary statistics were presented for these two explorations, with no statistical inference. The association of baseline factors with adherence and perceived relaxation categories was presented.

Basic demographics of the recruited participants were compared with aggregate data from the potentially eligible population with no statistical inferences made.

Primary analysis took place on a complete case basis. If more than 10% of primary outcome data were missing, sensitivity analysis was planned to explore the pattern of missingness for the primary outcome, using multiple imputation and/or a pattern mixture model as appropriate. Multiple imputation took into account missing data across the three timepoints that could contribute to the primary outcome, not just present or missing primary outcome. Sensitivity analyses also assessed whether the complex definition of the primary outcome had led to bias by assessing the impact of using day 21 expressing yield only, and separately of excluding day four logs. Stata v18.0 was used for all analysis.

Post-hoc meta-regression was performed after including the adjusted primary outcome from this study with the other studies described in Chapter 3 (Figure 3.3), and regressing the standardised effect estimate against the mean gestational age at birth of infants included in each study. Random effects meta-regression was performed using the `metareg` command within Stata 18.0. This used a variance-weighted least squares technique, where the regression was weighted according to the inverse of each study's variance. The study variance was calculated using the standard deviation and an estimate of between-study variance. Residual maximum likelihood was used to estimate the between-study variance. The Knapp-Hartung correction was applied to the meta-regression results. Sensitivity analysis was performed using only studies of milk expression, i.e. excluding studies of direct breastfeeding.

#### **5.2.13 Participant confidentiality and retention of personal data**

The Participant Information Sheet provided the required information about data processing. Participant contact details were necessary to send CRFs and SMS. They were kept in an administrative database, linked to the clinical database by study identifier. All electronic databases complied with relevant security standards and were regularly backed up. Identifiable information will be kept centrally for a period of five years and then reviewed. All data kept centrally will be archived according to Clinical Trials Unit standard operating procedures.

#### **5.2.14 Participant remuneration**

Each participant was provided with a small digital weighing scale, a cool bag and a pen. They were offered a set of headphones if in the intervention group. Due to higher than expected loss to follow up for the primary outcome, an amendment was approved after recruitment started to provide a £10 electronic food and drink voucher code to participants during the expressing phase. This was an unconditional thank you gift for the participant with the aim to increase the participant's feeling of connection with and positivity about the trial.

### **5.3 Ethical considerations**

The trial was run in the context of families experiencing the trauma of very preterm birth. Many infants were very unwell, or perceived to be very unwell by their families even when their clinical course was smooth. It was known that some infants would die, including one of a multiple birth where the other survived. Much of the recruitment period took place at a time when parents were likely to be further affected by the Covid-19 pandemic, both in their lives outside of the neonatal unit and in terms of potential increased separation from and anxiety about their baby. The participants of the trial were therefore under major stress throughout the period of the trial, which mandated a sensitive and careful approach to study design.

Parent involvement described in Chapter 4 ensured that the trial structure and the language of all trial documents took this situation into account. Infant deaths were frequently monitored during the trial period to ensure we would not ask mothers for research data at the time of their bereavement.

If the participants' scores on the mental health assessments at day 21 exceeded a pre-specified threshold for concern, the participant was sent information on self-help and how to access individualised neonatal unit-based psychological support.

### **5.4 Summary**

This chapter has described the methods of the randomised controlled trial forming the major programme of work for this thesis. The next chapter will present the results of the analyses described above, followed by a subsequent chapter describing the pre-planned exploratory analysis.

# Chapter 6: Randomised Trial Results

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## 6.1 Chapter Overview

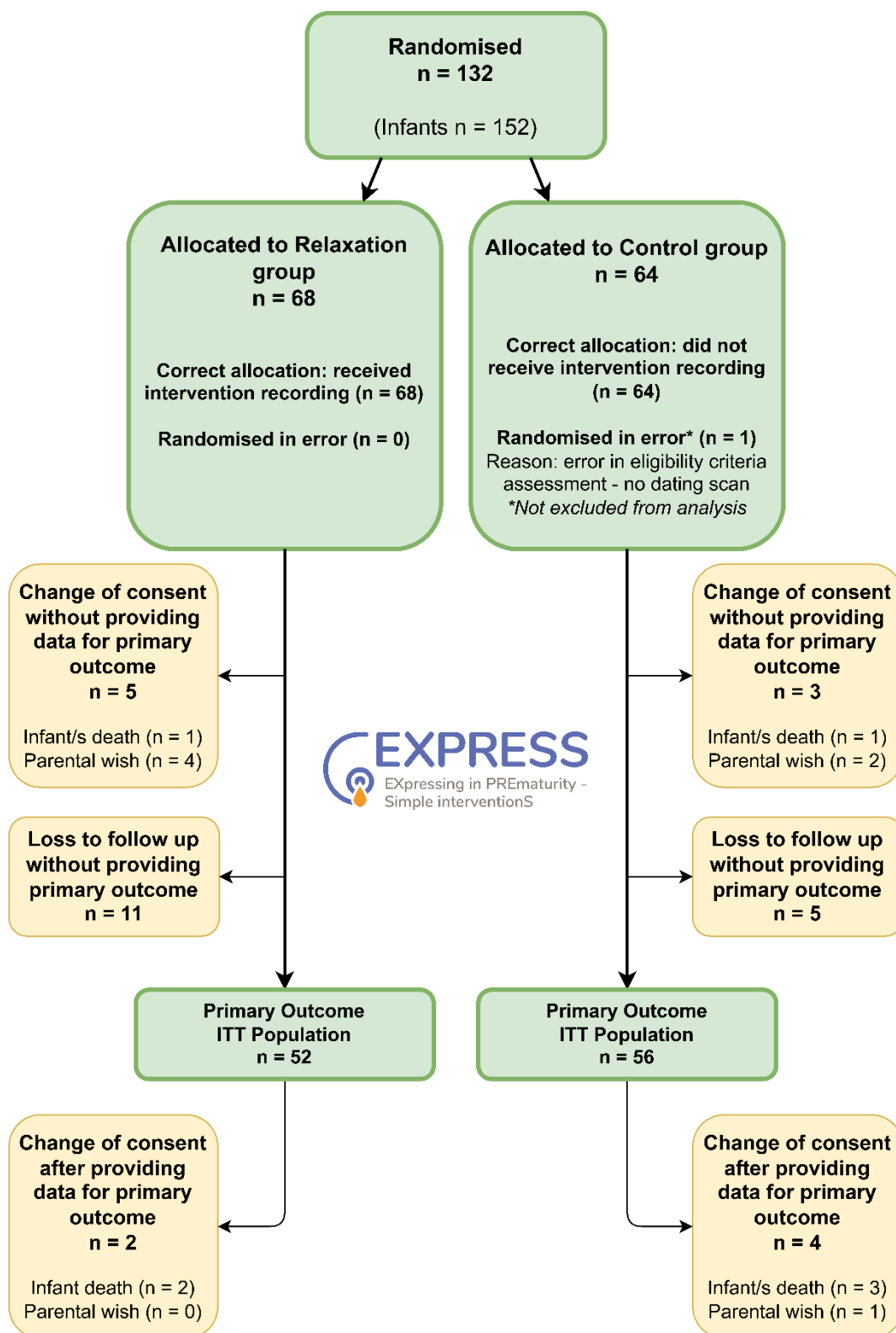
The previous chapter outlined the methods to carry out a randomised controlled trial of a relaxation intervention in mothers of very preterm infants. This chapter reports the results of the randomised comparison and its implications. Much of the research presented in this chapter has been submitted for publication. I am the lead author of the submitted paper. The co-authors are the doctoral supervisors and Clinical Trials Unit staff within the National Perinatal Epidemiology Unit. I carried out the majority of the recruitment described below and performed all the statistical analysis. The co-authors supported the design of the research, administration and logistical support for trial processes, oversight and independent verification of the primary outcome.

## 6.2 Trial Process

Recruitment was conducted from August 2<sup>nd</sup> 2021 to October 31<sup>st</sup> 2022; follow up was completed in January 2023. Figure 6.1 shows the CONSORT flowchart; 68 participants were allocated to the relaxation group and 64 to the control group. For the primary outcome, there was minimal disparity in missing data due to participant change of consent, including infant death (5 in the relaxation group and 3 in the control group). There was some disparity in loss to follow up (11 in the relaxation group and 5 in the control group). Data completeness (shown later in Table 6.6 and Table 6.9) was highest for the primary outcome (82%) and the feeding assessment at 36 weeks' PMA (86%).

Appendix E provides a summary of the evidence for mitigation or realisation of potential risks to trial participants and trial integrity that were identified before and during trial recruitment.

Figure 6.1: CONSORT flowchart





### 6.3 Baseline characteristics

Table 6.1 and Table 6.2 show the baseline characteristics of the study population, by allocation arm and overall. The majority of participants were recruited on day 2 and 3 after birth (69.7%, 92/132). Half had given birth at less than 28 weeks' PMA (53.8%, 71/132) and a quarter at less than 26 weeks' PMA (25.8%, 34/132).

In terms of ethnic background, 72 (59.5%) participants were White, 21 (17.4%) were Black and 22 (18.2%) Asian. The mean age was 32.8 years (SD 6.3). Overall, 24 participants (18.3%) lived in the most deprived quintile of English postcodes and 28 (21.4%) lived in the least deprived quintile. When asked at baseline, 85 (70.2%) had an intention to exclusively breastmilk feed at discharge home. Using a STAI threshold of 40 or more, 80 participants (72.7%) had clinical anxiety at baseline.

**Table 6.1: Baseline participant characteristics of the study population**

|                                                                  | Relaxation Group (n = 68) | Control Group (n = 64) | Total (n = 132) |
|------------------------------------------------------------------|---------------------------|------------------------|-----------------|
| <b>Ethnic background, n (%)</b>                                  |                           |                        |                 |
| White                                                            | 38 (64.4)                 | 34 (54.8)              | 72 (59.5)       |
| Asian or Asian British                                           | 8 (13.6)                  | 14 (22.6)              | 22 (18.2)       |
| Black, African, Black British or Caribbean                       | 10 (16.9)                 | 11 (17.7)              | 21 (17.4)       |
| Mixed or multiple ethnic groups                                  | 0                         | 3 (4.8)                | 3 (2.5)         |
| Other                                                            | 2 (3.4)                   | 0                      | 2 (1.7)         |
| Prefer not to say                                                | 1 (1.7)                   | 0                      | 1 (0.8)         |
| Missing                                                          | 9                         | 2                      | 11              |
| <b>Age (years), mean (SD)</b>                                    | 33.1 (6.5)                | 32.4 (6.2)             | 32.8 (6.3)      |
| Median [IQR]                                                     | 33 [30 to 36]             | 33 [28 to 35]          | 33 [29 to 36]   |
| (Min to max)                                                     | (19 to 55)                | (21 to 54)             | (19 to 55)      |
| <b>Index of Multiple Deprivation quintile<sup>8</sup>, n (%)</b> |                           |                        |                 |
| 1 (Most deprived)                                                | 15 (22.1)                 | 9 (14.3)               | 24 (18.3)       |
| 2                                                                | 9 (13.2)                  | 17 (27.0)              | 26 (19.8)       |
| 3                                                                | 11 (16.2)                 | 11 (17.5)              | 22 (16.8)       |
| 4                                                                | 15 (22.1)                 | 16 (25.4)              | 31 (23.7)       |
| 5 (Least deprived)                                               | 18 (26.5)                 | 10 (15.9)              | 28 (21.4)       |
| Missing                                                          | 0                         | 1                      | 1               |
| <b>Age at leaving full-time education, n (%)</b>                 |                           |                        |                 |
| 16 years old or less                                             | 5 (8.5)                   | 9 (14.5)               | 14 (11.6)       |
| 17 or 18 years old                                               | 11 (18.6)                 | 13 (21.0)              | 24 (19.8)       |
| 19 years old or more                                             | 38 (64.4)                 | 39 (62.9)              | 77 (63.6)       |
| Still in full time education                                     | 4 (6.8)                   | 1 (1.6)                | 5 (4.1)         |
| Prefer not to say                                                | 1 (1.7)                   | 0                      | 1 (0.8)         |
| Missing                                                          | 9                         | 2                      | 11              |

<sup>8</sup>Assigned by postcode to create comparable classification areas of similar deprivation level across the country

|                                                                                      | <b>Relaxation Group (n = 68)</b> | <b>Control Group (n = 64)</b> | <b>Total (n = 132)</b> |
|--------------------------------------------------------------------------------------|----------------------------------|-------------------------------|------------------------|
| <b>Lives with a partner, n (%)</b>                                                   | 54 (91.5)                        | 54 (87.1)                     | 108 (89.3)             |
| Missing                                                                              | 9                                | 2                             | 11                     |
| <b>Current smoker, n (%)</b>                                                         | 4 (6.8)                          | 4 (6.5)                       | 8 (6.6)                |
| Missing                                                                              | 9                                | 2                             | 11                     |
| <b>Intensive care in the 48hr after birth, n (%)</b>                                 | 1 (1.5)                          | 0 (0.0)                       | 1 (0.8)                |
| <b>Time from birth to first expression of milk (hours), median (IQR)</b>             | 6 [2 to 12]                      | 6 [3 to 12]                   | 6 [3 to 12]            |
| (Min to max)                                                                         | (0 to 96)                        | (0 to 72)                     | (0 to 96)              |
| Missing                                                                              | 9                                | 3                             | 12                     |
| <b>Intention for exclusive breastmilk at discharge<sup>9</sup>, n (%)</b>            | 41 (69.5)                        | 44 (71.0)                     | 85 (70.2)              |
| Missing                                                                              | 9                                | 2                             | 11                     |
| <b>STAI<sup>10</sup> score at randomisation, mean (SD) (score range 20 - 80)</b>     | 53.5 (14.4)                      | 50.2 (14.0)                   | 51.8 (14.2)            |
| Median [IQR]                                                                         | 50 [43 to 67]                    | 50 [40 to 60]                 |                        |
| (Min to max)                                                                         | (20 to 80)                       | (20 to 80)                    | (20 to 80)             |
| Score >40                                                                            | 43 (78.2)                        | 37 (67.3)                     | 80 (72.7)              |
| Missing                                                                              | 13                               | 9                             | 22                     |
| <b>Mode of delivery<sup>11</sup>, n (%)</b>                                          |                                  |                               |                        |
| Vaginal birth                                                                        | 25 (36.8)                        | 32 (50.0)                     | 57 (43.2)              |
| Instrumental birth                                                                   | 0 (0.0)                          | 0 (0.0)                       | 0 (0.0)                |
| Caesarean birth                                                                      | 43 (63.2)                        | 32 (50.0)                     | 75 (56.8)              |
| Missing                                                                              | 0                                | 0                             | 0                      |
| <b>Multiple pregnancy<sup>12</sup>, n (%)</b>                                        | 11 (16.2)                        | 9 (14.1)                      | 20 (15.2)              |
| <b>Primiparous, n (%)</b>                                                            | 37 (59.7)                        | 37 (59.7)                     | 74 (59.7)              |
| Missing                                                                              | 6                                | 2                             | 8                      |
| <b>Multipara only, N</b>                                                             | 25                               | 25                            | 50                     |
| <b>Any previous breastmilk feeding experience, n (%)</b>                             | 23 (95.8)                        | 21 (84.0)                     | 44 (88.0)              |
| Missing                                                                              | 1                                | 0                             | 1                      |
| <b>Length of previous breastmilk feeding experience (weeks), median (IQR); n (%)</b> | 52 [17 to 79]                    | 32 [10 to 52]                 | 39 [14 to 62]          |
| < 1 week                                                                             | 0 (0.0)                          | 0 (0.0)                       | 0 (0.0)                |
| 1 week to < 6 weeks                                                                  | 3 (13.0)                         | 2 (9.5)                       | 5 (11.0)               |
| 6 weeks to < 6 months                                                                | 5 (21.7)                         | 8 (38.1)                      | 13 (30.0)              |
| 6 months to < 12 months                                                              | 4 (17.4)                         | 5 (23.8)                      | 9 (20.0)               |
| ≥ 12 months                                                                          | 11 (47.8)                        | 6 (28.6)                      | 17 (39.0)              |
| Missing                                                                              | 2                                | 4                             | 6                      |

Missing data are excluded from percentage denominators

<sup>9</sup>Defined as “your baby would be drinking only your breastmilk and no infant formula”

<sup>10</sup>Six item STAI, transformed to an equivalent score to 20 item STAI

<sup>11</sup>If two infants, the most invasive method of birth is reported, where caesarean birth is more invasive than instrumental birth and instrumental birth is more invasive than unassisted vaginal birth

<sup>12</sup>Stratification factor

**Table 6.2: Baseline infant characteristics of the study population**

|                                                                             | Relaxation Group (n = 68) | Control Group (n = 64) | Total (n = 132) |
|-----------------------------------------------------------------------------|---------------------------|------------------------|-----------------|
| <b>Recruiting centre<sup>13</sup>, n (%)</b>                                |                           |                        |                 |
| 1                                                                           | 39 (57.4)                 | 38 (59.4)              | 77 (58.3)       |
| 2                                                                           | 17 (25.0)                 | 15 (23.4)              | 32 (24.2)       |
| 3                                                                           | 5 (7.4)                   | 5 (7.8)                | 10 (7.6)        |
| 4                                                                           | 7 (10.3)                  | 6 (9.4)                | 13 (9.8)        |
| <b>Born in recruiting centre, n (%)</b>                                     | 66 (97.1)                 | 57 (89.1)              | 123 (93.2)      |
| <b>Postnatal age at randomisation (days), n (%)</b>                         |                           |                        |                 |
| Day 0 (day of birth)                                                        | 2 (2.9)                   | 5 (7.8)                | 7 (5.3)         |
| Day 1                                                                       | 14 (20.6)                 | 19 (29.7)              | 33 (25)         |
| Day 2                                                                       | 30 (44.1)                 | 18 (28.1)              | 48 (36.4)       |
| Day 3                                                                       | 22 (32.4)                 | 22 (34.4)              | 44 (33.3)       |
| <b>Gestational age at birth (weeks)<sup>14</sup>, mean (SD)</b>             | 27.8 (2.4)                | 27.8 (2.4)             | 27.8 (2.4)      |
| Median [IQR]                                                                | 28 [26 to 30]             | 28 [26 to 30]          | 28 [26 to 30]   |
| (Min to Max - rounded)                                                      | (23 to 32)                | (23 to 32)             | (23 to 32)      |
| <b>Gestational age at birth (weeks), n (%)</b>                              |                           |                        |                 |
| 23 to < 26 weeks                                                            | 19 (27.9)                 | 15 (23.4)              | 34 (25.8)       |
| 26 to < 28 weeks                                                            | 17 (25.0)                 | 20 (31.3)              | 37 (28.0)       |
| 28 to < 30 weeks                                                            | 15 (22.1)                 | 14 (21.9)              | 29 (22.0)       |
| 30 to < 32 weeks                                                            | 17 (25.0)                 | 15 (23.4)              | 32 (24.2)       |
| <b>Apgar score at 5 minutes<sup>15</sup>, n (%)</b>                         |                           |                        |                 |
| Median [IQR]                                                                | 8 [7 to 10]               | 9 [7 to 10]            | 9 [7 to 10]     |
| <5                                                                          | 4 (6.1)                   | 1 (1.7)                | 5 (4.0)         |
| Missing                                                                     | 2                         | 4                      | 6               |
| <b>Ventilated at randomisation (at least one infant if multiple), n (%)</b> | 23 (33.8)                 | 24 (37.5)              | 47 (35.6)       |

Missing data are excluded from percentage denominators

## 6.4 Comparison of trial participants to the screening population

Trial participants had a similar age, birth gestation, mode of birth and rate of multiple pregnancy to the population eligible for screening, as reported in anonymised routinely collected data (Table 6.3). There was some disparity in participant parity. In the screening population, 165/341 (48.5%) were primiparous compared with 74/124 (59.7%) of trial recruits.

<sup>13</sup>Stratification factor

<sup>14</sup>Stratification factor

<sup>15</sup>If two infants, the lower Apgar score of the two

**Table 6.3: Comparison of recruited and potentially eligible population**

|                                        | <b>Trial participants<br/>(n = 132)</b> | <b>Population eligible for<br/>screening<sup>16</sup> (n = 381)</b> |
|----------------------------------------|-----------------------------------------|---------------------------------------------------------------------|
| <b>Gestational age at birth, n (%)</b> |                                         |                                                                     |
| 23+0 – 27+6                            | 71 (53.8)                               | 203 (53.3)                                                          |
| 28+0 – 31+6                            | 61 (46.2)                               | 178 (46.7)                                                          |
| <b>Primiparous, n (%)</b>              | 74 (59.7)                               | 165 (48.5)                                                          |
| Missing                                | 8                                       | 41                                                                  |
| <b>Multiple pregnancy, n (%)</b>       | 20 (15.2)                               | 54 (17.0)                                                           |
| Missing                                | 0                                       | 64                                                                  |
| <b>Mode of delivery, n (%)</b>         |                                         |                                                                     |
| Vaginal birth                          | 57 (43.2)                               | 157 (42.3)                                                          |
| Caesarean birth                        | 75 (56.8)                               | 214 (57.7)                                                          |
| Missing                                | 0                                       | 10                                                                  |
| <b>Mother's age (years), mean (SD)</b> | 32.8 (6.3)                              | 32.3 (6.8)                                                          |
| Median [IQR]                           | 33 [29 to 36]                           | 32 [27 – 36]                                                        |
| (Min to max)                           | (19 to 54)                              | (17 to 54)                                                          |
| Missing                                | 0                                       | 6                                                                   |

*Missing data are excluded from percentage denominators*

## 6.5 Adherence to and perception of the intervention

Participants in the relaxation group who provided data, reported high levels of adherence. On day 4, 14 and 21 the median listening frequency was 3 times per day (Table 6.4) and 98.1% of those who provided data, reported ever listening to the study recording (51/52). There was some use of relaxation in the control group; 6.8% (3/44) listened to or practiced non-intervention relaxation material or techniques daily or more, and an additional 13.6% (6/44) did so more than once a week. More participants in the relaxation group (64.3%, 27/42) had practiced non-intervention relaxation in the study period than in the control group (25.0%, 11/44).

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<sup>16</sup>Anonymised data was extracted from a prospectively entered clinical database (Badgernet, Clevermed). Information related to twin one was used for multiple births, to represent the mother. This population includes trial participants, mothers who declined participation, mothers who were not eligible to consent after full screening and mothers who were not approached but fell into the appropriate gestational age range with one or two infants, and present in a recruiting hospital within the first three days after birth

**Table 6.4: Participant relaxation characteristics**

|                                                       | Relaxation Group<br>(n = 68) | Control Group<br>(n = 64) |
|-------------------------------------------------------|------------------------------|---------------------------|
| Frequency of listening to intervention (times/day)    |                              |                           |
| Day 4, mean (SD)                                      | 3.8 (3.3)                    |                           |
| Median [IQR]                                          | 3 [1 to 6]                   |                           |
| Missing                                               | 25                           |                           |
| Day 14, mean (SD)                                     | 3.5 (3.0)                    |                           |
| Median [IQR]                                          | 3 [1 to 5]                   |                           |
| Missing                                               | 26                           |                           |
| Day 21, mean (SD)                                     | 3.0 (2.5)                    |                           |
| Median [IQR]                                          | 3 [1 to 5]                   |                           |
| Missing                                               | 26                           |                           |
| Ever reported listening to recording, n (%)           | 51 (98.1)                    |                           |
| Missing                                               | 16                           |                           |
| Non-intervention relaxation, n (%)                    |                              |                           |
| Daily or more                                         | 3 (7.1)                      | 3 (6.8)                   |
| More than once a week, less than once a day           | 8 (19.0)                     | 6 (13.6)                  |
| Once a week or less                                   | 16 (38.1)                    | 2 (4.5)                   |
| Not at all                                            | 15 (35.7)                    | 33 (75.0)                 |
| Missing                                               | 26                           | 20                        |
| Perception of intervention on day 21, n (%)           |                              |                           |
| Like/very much like                                   | 25 (59.5)                    |                           |
| Neutral                                               | 12 (28.6)                    |                           |
| Dislike/very much dislike                             | 5 (11.9)                     |                           |
| Missing                                               | 26                           |                           |
| Perception of effect of intervention on day 21, n (%) |                              |                           |
| More relaxed/much more relaxed                        | 32 (76.2)                    |                           |
| Neutral                                               | 6 (14.3)                     |                           |
| Less relaxed/much less relaxed                        | 4 (9.5)                      |                           |
| Missing                                               | 26                           |                           |

*Missing data are excluded from percentage denominators*

The majority of relaxation group participants who provided data, liked the study recording (25/42, 59.5%) and felt that it was relaxing (32/42, 76.2%). However, 12% of those who responded disliked or very much disliked it (5/42).

Table 6.5 shows the latest chronological free text submission provided by the 32 relaxation group participants who commented on the recording. Two thirds of these participants gave positive comments at some point during the study period, related to feeling relaxed, calm or re-framing traumatic birth (22 participants). In terms of particularly positive experiences, the following comments are notable:

*“When I start listening the recording [sic] and meditating before expressing, milk starts falling down my chest from both breasts without me touching at all” - participant 72*

*“because my birth and recovery has felt rather traumatic, the recording reminds me to focus on the things that I can do right now and being present while I’m expressing focusing on the love I have for my child as opposed to all the other unsavoury bits” – participant 112*

A similar number gave negative comments at some point during the study period (20 in total, some of whom also gave positive comments). These related to the monotony of listening to the same recording (six participants), dislike of recording content (seven participants) or length (five participants), and difficulty finding time to listen (seven participants). In terms of potential harms to participants the following comments are notable:

*“...bits about holding baby and talking to baby when I can't feel almost cruel... a bit antagonising” (participant 2)*

*“I feel pressure about listening to the recording, and I don't really like listening to it” (participant 47)*

*“The first time I listened to it, the parts about imagining your baby nuzzling the breast, or holding your baby, made me feel a bit sad because that's really far off in our situation” (participant 106)*

**Table 6.5: Qualitative comments about the experience of using the intervention recording**

| ID | Day    | Response to “Do you have any comments about the study recording?”                                                                                                                                                                                                                                               |
|----|--------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2  | Day 21 | I have started to find I stop 'listening' but enjoy how the voice washes over me. I still find the comment about learning to be a mother irritates me and the mention of a to-do list makes me think about everything I need to do when I haven't before!                                                       |
| 4  | Day 21 | Find it difficult to listen to the recording as in the hospital so much                                                                                                                                                                                                                                         |
| 11 | Day 4  | The recording helps to relax when expressing in different environments. It allows me to block out the noise and distractions with the breathing exercises. I focus less on the amount I am expressing and I have been able to express for longer. The recording could be a bit longer but I can always restart. |
| 16 | Day 21 | Nothing other than it would be nice to have some variety.                                                                                                                                                                                                                                                       |
| 23 | Day 14 | It's good, I just wouldn't listen to it all the time as it can get quite repetitive                                                                                                                                                                                                                             |
| 26 | Day 21 | As I have heard the study recording quite a few times now, it can feel a bit repetitive. I feel quite relaxed when I begin listening to it, but as I get towards the middle/end I find myself getting more distracted now.                                                                                      |
| 28 | Day 21 | Needed to be longer personally                                                                                                                                                                                                                                                                                  |
| 33 | Day 4  | It could be a little longer                                                                                                                                                                                                                                                                                     |
| 34 | Day 4  | I'd like it if it was a bit longer                                                                                                                                                                                                                                                                              |
| 39 | Day 14 | If [baby] is having a good day the recording is brilliant i can actually pay attention to it when we have a day which isnt as good i find it hard to concentrate on it, it becomes more of a chore than a relaxation method.                                                                                    |
| 41 | Day 21 | Finding time to remember to listen is tricky                                                                                                                                                                                                                                                                    |
| 42 | Day 4  | It's helping calm me whilst I feed                                                                                                                                                                                                                                                                              |
| 47 | Day 21 | I feel pressure about listening to the recording, and I don't really like listening to it (sorry)                                                                                                                                                                                                               |
| 53 | Day 4  | This study has really helped keep me calm and keep my mind off things                                                                                                                                                                                                                                           |
| 54 | Day 21 | It is boring to listen to the same thing over and over again.                                                                                                                                                                                                                                                   |

| ID  | Day    | Response to “Do you have any comments about the study recording?”                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|-----|--------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 59  | Day 14 | Struggle at times to find quite [quiet] space to express, especially at hospital due to either bedside expressing or room with curtain separating from kitchen area.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| 60  | Day 4  | Good                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| 62  | Day 4  | I think I would prefer calming music rather than someone talking. I feel this would relax [relax] me a lot more                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 72  | Day 21 | I think the recording is very helpful                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| 87  | Day 21 | It could be longer                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| 89  | Day 21 | I would like to do it with some background music or natural world sounds                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| 91  | Day 14 | So far I think the recording could be helpful sometimes but for me I feel like I express more milk when I am with my baby or I am looking at a picture of him. I feel less stressed. The recording helps you relax but I don't know if it affects how much I'm expressing.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| 98  | Day 21 | Excellent                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| 106 | Day 21 | Found it a bit harder to focus on this week, perhaps because I've now listened so many times! On stressful days it definitely makes me more calm. It's a good length - enough to relax but not too long, once it finishes I pay a bit more attention to expressing & any areas that need a massage etc.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| 111 | Day 21 | Nice relaxing voice                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| 112 | Day 14 | The recording is still helpful. I believe because my birth and recovery has felt rather traumatic, the recording reminds me to focus on the things that I can do right now and being present while I'm expressing focusing on the love I have for my child as opposed to all the other unsavoury bits.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| 117 | Day 14 | It's good                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| 118 | Day 14 | It's good for relaxing                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| 121 | Day 21 | It really good to listen to because it makes you relaxed.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| 126 | Day 21 | I still find it difficult to fully listen to the recording - my mind flicks to other thoughts frequently but I do find my mind is always back on the recording by the end (e.g. for the count down). After 3 weeks of listening it would be good to have a slightly different version of it - perhaps versions that focus on different aspects of relaxation combined with thoughts of your baby (e.g. more on breathing exercises, more on relaxing body parts) or versions that you could listen to whilst driving to the hospital as even though you are not pumping at the time it helps get through a more stressful part of the day. This week I've had quite a few issues with blocked ducts so it would be good to have a part at the start that perhaps focuses on massage – because [because] I never make time to do this before pumping. |
| 130 | Day 4  | The recording is relaxing - but listening to the same recording every time is very repetitive and I think would put me off long term.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| 132 | Day 14 | I haven't used the recording as it makes no sense too and have no quiet time                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |

ID = participant sequential number (not the study identifier used for data analysis). Where a participant submitted more than one comment, the latest chronological comment is reported here. Presented as written by participants. Infant name replaced by [baby]. Suggested clarifications in square brackets

## 6.6 Primary outcome

There was no evidence of a difference in the primary outcome of highest 24-hour milk quantity on any of day 4, 14 or 21 (adjusted mean difference 73.9g, 95% CI -61.7 to 209.5g; Table 6.6). Fewer participants submitted day 4 and 14 logs in the relaxation group (day 4 relaxation group: 72%, 49/68; control group: 81%, 52/64). Similar numbers submitted day 21 logs (relaxation group: 69%,

47/68; control group: 67%, 43/64). Thus the primary outcome was formed by the day 4 log more often in the control group than the relaxation group (relaxation group: 15%, 8/52; control group: 25%, 14/56). Appendix Table F.5 shows that the pre-specified sensitivity analyses addressing the possibility of unequal timepoint measurement produced a similar effect size estimate to the adjusted mean difference (which includes adjustment for measurement day).

**Table 6.6: Primary outcome and associated factors**

|                                                        | Relaxation Group (n = 68) | Control Group (n = 64) | Unadjusted MD (95% CI) | Adjusted MD (95% CI) <sup>17</sup> | p value (adjusted) |
|--------------------------------------------------------|---------------------------|------------------------|------------------------|------------------------------------|--------------------|
| Highest 24-hour milk weight on day 4, 14 or 21 (grams) |                           |                        |                        |                                    |                    |
| Mean (SD)                                              | 596.7 (433.6)             | 467.7 (350.2)          | 129.1 (-20.8 to 278.9) | 73.9 (-61.7 to 209.5)              | 0.282              |
| Median [IQR]                                           | 521 [255 to 903]          | 398 [177 to 719]       |                        |                                    |                    |
| Min to max                                             | (2 to 1926)               | (7 to 1274)            |                        |                                    |                    |
| Missing                                                | 16                        | 8                      |                        |                                    |                    |
| Data available, n (%):                                 |                           |                        |                        |                                    |                    |
| Day 4                                                  | 49 (72.1)                 | 52 (81.3)              |                        |                                    |                    |
| Day 14                                                 | 45 (66.2)                 | 45 (70.3)              |                        |                                    |                    |
| Day 21                                                 | 47 (69.1)                 | 43 (67.2)              |                        |                                    |                    |
| Data used in primary outcome, n (%):                   |                           |                        |                        |                                    |                    |
| Day 4                                                  | 8 (15.4)                  | 14 (25.0)              |                        |                                    |                    |
| Day 14                                                 | 11 (21.2)                 | 15 (26.8)              |                        |                                    |                    |
| Day 21                                                 | 33 (63.5)                 | 27 (48.2)              |                        |                                    |                    |

MD = mean difference. SD = standard deviation. IQR = interquartile range. CI = confidence intervals.

Appendix Table F.6 shows measures of data quality. The majority of logs were submitted within 48 hours of the requested timepoint (99.3%, 284/286). There was evidence of erroneous recording in 6.3% (18/286) of all logs (where the milk and container weight was recorded as less than the container weight).

## 6.7 Exploratory subgroup analyses

There was no evidence of an interaction between intervention allocation and gestational age group ( $p=0.74$ ). The adjusted mean difference in the primary outcome was  $53.0 \pm 184.6\text{g}$  and  $100.0 \pm 199.4\text{g}$  in mothers of extremely preterm and very preterm infants respectively, demonstrating that

<sup>17</sup>Adjusted for multiple birth, gestation at birth, centre and measurement day



the direction of the data was towards a lower effect estimate in mothers who gave birth at lower gestations.

No comparative statistics are presented for the remaining outcomes and subgroups (Table 6.7). Although the difference in milk quantity for mothers of twins appears large, the number of mothers with twins was small (11 in the relaxation group and 9 in the CG) and one participant with twins in the relaxation group was an outlier in terms of expressed yield.

**Table 6.7: Exploratory sub-group analyses**

|                                                             |              | Relaxation Group<br>(n = 68) | Control Group<br>(n = 64) | Adjusted MD<br>(95% CI) <sup>18</sup> | p value |
|-------------------------------------------------------------|--------------|------------------------------|---------------------------|---------------------------------------|---------|
| Sub-group 1: Gestational age at birth (weeks)               |              |                              |                           |                                       |         |
| 24-hour milk weight (g)                                     |              |                              |                           |                                       |         |
| 23+0 to 27+6                                                | Mean (SD)    | 585.8 (489.0)                | 450.5 (381.9)             | 53.0 (-131.6 to 237.6)                | 0.73    |
|                                                             | Median [IQR] | 546 [101 to 942]             | 326 [71 to 745]           |                                       |         |
| 28+0 to 31+6                                                | Mean (SD)    | 609.4 (368.5)                | 488.9 (312.8)             | 100.0 (-99.3 to 299.4)                |         |
|                                                             | Median [IQR] | 516 [304 to 847]             | 491 [243 to 678]          |                                       |         |
| Missing (n)                                                 |              | 16                           | 8                         |                                       |         |
| Exclusive breastmilk at 36 weeks' PMA, n (%)                |              |                              |                           |                                       |         |
| 23+0 – 27+6                                                 |              | 18 (58.1)                    | 18 (62.1)                 |                                       |         |
| 28+0 – 31+6                                                 |              | 19 (70.4)                    | 19 (73.1)                 |                                       |         |
| Missing                                                     |              | 10                           | 9                         |                                       |         |
| Any breastmilk at 36 weeks' PMA, n (%)                      |              |                              |                           |                                       |         |
| 23+0 – 27+6                                                 |              | 26 (83.9)                    | 23 (79.3)                 |                                       |         |
| 28+0 – 31+6                                                 |              | 25 (92.6)                    | 24 (92.3)                 |                                       |         |
| Missing                                                     |              | 10                           | 9                         |                                       |         |
| Subgroup 2: Intention for exclusive breastmilk at discharge |              |                              |                           |                                       |         |
| Missing for feeding intention (n)                           |              | 9                            | 2                         |                                       |         |
| 24-hour milk weight (g)                                     |              |                              |                           |                                       |         |
| Yes                                                         | Mean (SD)    | 622.3 (485.8)                | 520.5 (370.3)             |                                       |         |
|                                                             | Median [IQR] | 572 [199 to 1042]            | 503 [211 to 810]          |                                       |         |
| No/unsure                                                   | Mean (SD)    | 539.2 (288.6)                | 355.8 (254.6)             |                                       |         |
|                                                             | Median [IQR] | 521 [341 to 718]             | 322 [157 to 653]          |                                       |         |
| Missing (n)                                                 |              | 7                            | 7                         |                                       |         |
| Exclusive breastmilk at 36 weeks' PMA, n (%)                |              |                              |                           |                                       |         |
| Yes                                                         |              | 27 (69.2)                    | 28 (73.7)                 |                                       |         |
| No/unsure                                                   |              | 8 (57.1)                     | 8 (50.0)                  |                                       |         |
| Missing                                                     |              | 6                            | 8                         |                                       |         |
| Any breastmilk at 36 weeks' PMA, n (%)                      |              |                              |                           |                                       |         |
| Yes                                                         |              | 34 (87.2)                    | 33 (86.8)                 |                                       |         |
| No/Unsure                                                   |              | 13 (92.9)                    | 13 (81.3)                 |                                       |         |
| Missing                                                     |              | 6                            | 8                         |                                       |         |

<sup>18</sup> Adjusted for multiple birth, gestation at birth, centre and measurement day

|                                                     |              | Relaxation Group<br>(n = 68) | Control Group<br>(n = 64) | Adjusted MD<br>(95% CI) <sup>18</sup> | p<br>value |
|-----------------------------------------------------|--------------|------------------------------|---------------------------|---------------------------------------|------------|
| <b>Sub-group 3: Multiple birth</b>                  |              |                              |                           |                                       |            |
| <b>24-hour milk weight (g)</b>                      |              |                              |                           |                                       |            |
| Singleton                                           | Mean (SD)    | 518.8 (377.8)                | 466.4 (356.2)             |                                       |            |
|                                                     | Median [IQR] | 481 [199 to 838]             | 398 [162 to 740]          |                                       |            |
| Multiple birth                                      | Mean (SD)    | 1025.2 (494.7)               | 475.2 (333.7)             |                                       |            |
|                                                     | Median [IQR] | 1118 [640 to 1221]           | 400 [279 to 674]          |                                       |            |
| Missing (n)                                         |              | 16                           | 8                         |                                       |            |
| <b>Exclusive breastmilk at 36 weeks' PMA, n (%)</b> |              |                              |                           |                                       |            |
| Singleton                                           |              | 31 (63.3)                    | 32 (68.1)                 |                                       |            |
| Multiple birth                                      |              | 6 (66.7)                     | 5 (62.5)                  |                                       |            |
| Missing                                             |              | 10                           | 9                         |                                       |            |
| <b>Any breastmilk at 36 weeks' PMA, n (%)</b>       |              |                              |                           |                                       |            |
| Singleton                                           |              | 42 (85.7)                    | 40 (85.1)                 |                                       |            |
| Multiple birth                                      |              | 9 (100)                      | 7 (87.5)                  |                                       |            |
| Missing                                             |              | 10                           | 9                         |                                       |            |

Missing data are excluded from percentage denominators. MD = mean difference. SD = standard deviation. IQR = interquartile range. CI = confidence intervals. PMA = post-menstrual age

## 6.8 Exploration of the primary outcome by adherence and perception of relaxation

Primary outcome data is presented in relation to the median listening frequency at the latest reported timepoint (Table 6.8). The method of categorisation of adherence was specified in the SAP (Appendix D). Within the relaxation group, higher adherence was associated with a higher adjusted primary outcome (adjusted mean difference 245.9g, 95% CI 36.0 to 455.7g,  $p=0.02$ ; adjusted for multiple birth, gestation at birth and centre as per primary outcome analysis; post-hoc use of statistical inference). There was no evidence to suggest that baseline or expressing-related variables were associated with adherence (Appendix Table F.7). There was no evidence of a difference in the primary outcome associated with perceived relaxation (Table 6.8).

**Table 6.8: Exploration of adherence and perception of relaxation with the primary outcome**

|                                            |              | Relaxation Group<br>(n=68)                    | Adjusted MD<br>(95% CI) <sup>19</sup> | p value |
|--------------------------------------------|--------------|-----------------------------------------------|---------------------------------------|---------|
| Adherence categories                       |              | Highest 24-hour milk weight <sup>20</sup> (g) |                                       |         |
| Higher adherence <sup>21</sup><br>(n = 21) | Mean (SD)    | 703.1 (395.3)                                 | 245.9<br>(36.0 to 455.7)              | 0.02    |
|                                            | Median [IQR] | 778 [346 to 1010]                             |                                       |         |
| Lower adherence <sup>22</sup><br>(n = 31)  | Mean (SD)    | 524.6 (449.5)                                 |                                       |         |
|                                            | Median [IQR] | 391 [170 to 687]                              |                                       |         |
| Missing primary outcome                    |              | 16                                            |                                       |         |
| Perception of intervention categories      |              | Highest 24-hour milk weight <sup>23</sup> (g) |                                       |         |
| Missing data for perception category       |              | 16                                            |                                       |         |
| Perceived as relaxing<br>(n = 40)          | Mean (SD)    | 609.8 (445.2)                                 |                                       |         |
|                                            | Median [IQR] | 571 [255 to 903]                              |                                       |         |
| Did not perceive as<br>relaxing (n = 11)   | Mean (SD)    | 625.7 (400.3)                                 |                                       |         |
|                                            | Median [IQR] | 599 [262 to 1010]                             |                                       |         |
| Missing primary outcome                    |              | 1                                             |                                       |         |

SD = standard deviation. IQR = interquartile range

## 6.9 Missing data

There was no evidence of differences in baseline characteristics for participants with present or absent primary outcome data (Appendix Table F.8). In contrast, for those participants who submitted all three logs compared to those who did not, there was a significant difference in recruiting trust; 67.5% of participants with complete logs were recruited in Oxford, in comparison to 44.2% of participants with incomplete logs ( $p=0.01$ ). Non-significant trends to other differences in baseline characteristics were that participants with three complete logs were more likely to have a partner and live in less deprived postcodes (Appendix Table F.9).

<sup>19</sup> Adjusted for multiple birth, gestation at birth, centre and measurement day

<sup>20</sup> On day 4, 14 or 21 after birth (primary outcome)

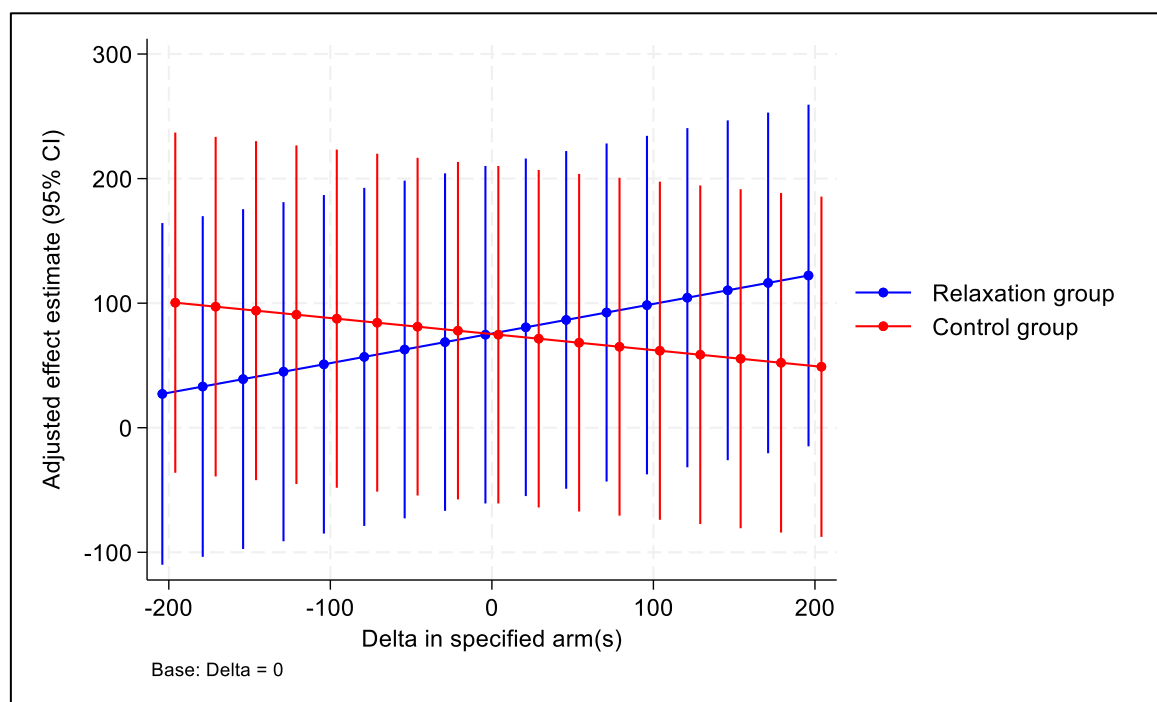
<sup>21</sup> Listening 3 or more times at last reported timepoint

<sup>22</sup> Listening 0-2 times at last reported timepoint; or has not responded to the question at any timepoint

<sup>23</sup> On day 4, 14 or 21 after birth (primary outcome)

In addition to the multiple imputation approach to missing data seen in Appendix Table F.5, Figure 6.2 presents the results of pattern mixture modelling. This shows that if missing primary outcome data were biased by up to 200g in either direction from the average, this would not change the conclusion of the analysis. The adjusted mean difference under these conditions ranged from 27.2g (95% CI -108.2 to 162.6) to 122.2g (95% CI -13.2 to 257.6).

**Figure 6.2: Modelling the effect of biased missing data by allocation arm**



A pattern mixture model is used on the adjusted primary outcome analysis with a range of effect sizes (delta) from -200g to +200g. This means that where the primary outcome is missing in the specified arm, it is imputed as the average value plus the delta value, to assess the effect of missing values being different to recorded values by up to 200g. Missing data in the other arm is imputed as the average value.

## 6.10 Secondary outcomes

There was no evidence of a difference in any secondary outcomes (Table 6.9). Of note, 24% of infant feeding status data at 36 weeks' (28/115) were taken from medical notes because of lack of SMS response. The first 30 cases where both SMS and medical notes were available, were checked to assess any discrepancy between the two sources of information on feeding status. There was 93% concordance.

**Table 6.9: Secondary outcomes**

|                                                                 | Relaxation Group<br>(n = 68) | Control Group<br>(n = 64) | Unadjusted effect estimate<br>(95% CI) | Adjusted effect estimate<br>(95% CI)          | p value |
|-----------------------------------------------------------------|------------------------------|---------------------------|----------------------------------------|-----------------------------------------------|---------|
| <b>Expressing ≥ 750g milk<sup>24</sup>, n (%)</b>               | 18 (34.6)                    | 12 (21.4)                 | RR 1.62<br>(0.86 to 3.02)              | RR 1.43<br>(0.77 to 2.66) <sup>25</sup>       | 0.26    |
| Missing                                                         | 16                           | 8                         |                                        |                                               |         |
| <b>Milk expressed (g/min), mean (SD)</b>                        | 4.5 (3.1)                    | 4.5 (3.6)                 | Med Diff -0.0<br>(-1.8 to 1.8)         | Med Diff 0.5<br>(-1.4 to 2.5) <sup>26</sup>   | 0.60    |
| Median [IQR]                                                    | 4 [2 to 6]                   | 4 [2 to 6]                |                                        |                                               |         |
| Missing                                                         | 25                           | 21                        |                                        |                                               |         |
| <b>STAI<sup>27</sup> score at day 21, mean (SD)</b>             | 48.3 (15.8)                  | 47.3 (15.9)               | MD 1.1<br>(-5.8 to 7.9)                | MD -1.9<br>(-8.2 to 4.3) <sup>28</sup>        | 0.54    |
| Median [IQR]                                                    | 47 [37 to 63]                | 47 [37 to 60]             |                                        |                                               |         |
| Score >40                                                       | 25 (58.1)                    | 23 (56.1)                 |                                        |                                               |         |
| Missing                                                         | 25                           | 23                        |                                        |                                               |         |
| <b>PCL-5 score<sup>29</sup> at day 21, mean (SD)</b>            | 19.4 (15.1)                  | 21.9 (16.8)               | Med Diff -2.0<br>(-10.9 to 6.9)        | Med Diff -1.4<br>(-11.8 to 9.0) <sup>26</sup> | 0.80    |
| Median [IQR]                                                    | 17 [10 to 25]                | 18 [9 to 33]              |                                        |                                               |         |
| Score >33                                                       | 6 (13.6)                     | 10 (22.7)                 |                                        |                                               |         |
| Missing                                                         | 24                           | 20                        |                                        |                                               |         |
| <b>Exclusive breastmilk at 36 weeks<sup>30</sup>, n (%)</b>     | 37 (63.8)                    | 37 (67.3)                 | RR 0.95<br>(0.72 to 1.24)              | RR 0.96<br>(0.74 to 1.24) <sup>26</sup>       | 0.75    |
| Missing                                                         | 10                           | 9                         |                                        |                                               |         |
| <b>Exclusive breastmilk at 4 months' CA<sup>27</sup>, n (%)</b> | 4 (13.3)                     | 4 (12.5)                  | RR 1.07<br>(0.29 to 3.89)              | RR 1.10<br>(0.30 to 4.01) <sup>31</sup>       | 0.89    |
| Not eligible <sup>32</sup>                                      | 25                           | 22                        |                                        |                                               |         |
| Missing                                                         | 16                           | 12                        |                                        |                                               |         |
| <b>Any breastmilk at 36 weeks<sup>33</sup>, n (%)</b>           | 51 (87.9)                    | 47 (85.5)                 | RR 1.03<br>(0.89 to 1.19)              | RR 1.03<br>(0.90 to 1.19) <sup>26</sup>       | 0.67    |
| Missing                                                         | 10                           | 9                         |                                        |                                               |         |

RR = relative risk; MD = mean difference. Med Diff = median difference. PMA = post-menstrual age. CA = corrected age. Missing data are excluded from percentage denominators

<sup>24</sup>Measured on day 4, 14 or 21

<sup>25</sup>Adjusted for multiple birth, gestation at birth, centre and measurement day

<sup>26</sup>Adjusted for multiple birth, gestation at birth and centre

<sup>27</sup>Six item Spielberger State-Trait Anxiety Index. Range is 20 – 80. Higher score represents more anxiety

<sup>28</sup>Adjusted for multiple birth, gestation at birth, centre and STAI at baseline

<sup>29</sup>Post-traumatic Stress Scale for DSM-5. Score range is 0 – 80, higher is more post-traumatic stress reactions

<sup>30</sup>No infant formula use in last 24 hours. If two infants, then applies to both

<sup>31</sup>Adjusted for gestation at birth

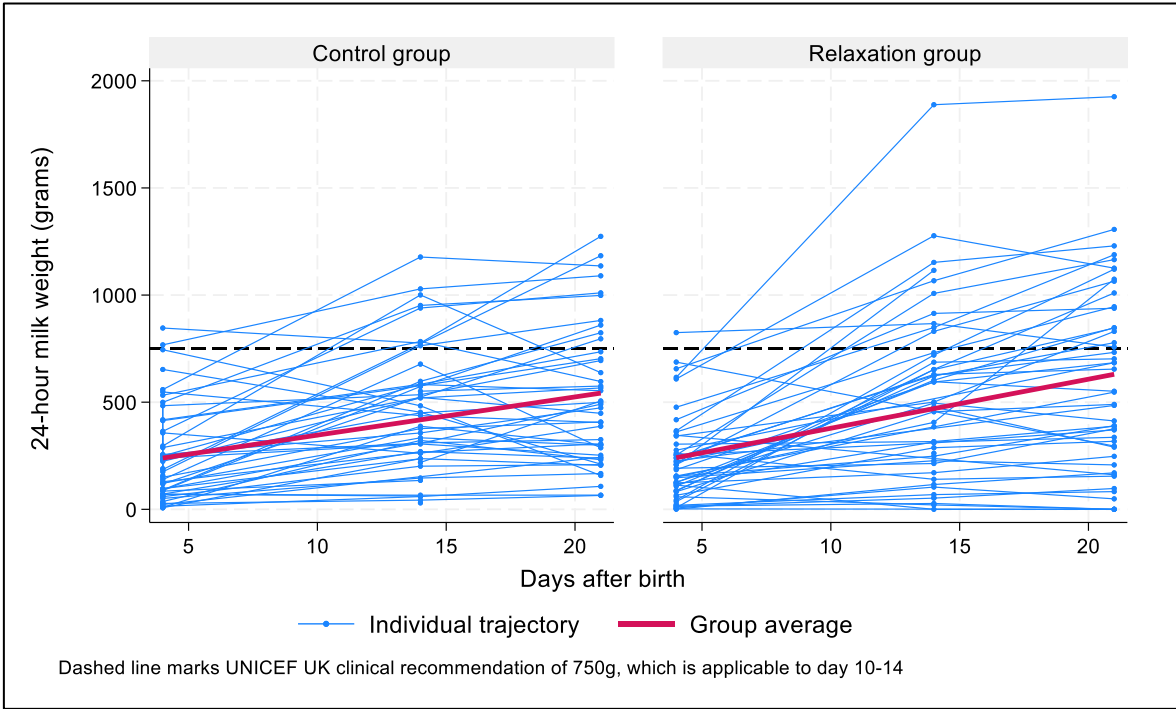
<sup>32</sup>These participants were recruited in the latter part of the study, which concluded at 36 weeks' PMA

<sup>33</sup>Defined as any breastmilk in the last 24 hours. If two infants, then any breastmilk for either infant

A notable non-significant trend in the secondary outcome data was that 13.6% of the relaxation group reported clinically important post-traumatic stress reactions at day 21 (6/44), compared to 22.7% of the control group (10/44).

Figure 6.3 shows participants' expressed milk yield trajectory in reference to the UNICEF UK BFI recommended 750g target (dashed line).

**Figure 6.3: Expressed milk yield trajectory over time since birth, by allocation arm**



### 6.11 Process indicators

There were no differences in any process indicators (Table 6.10 for data on day 21 and Appendix Table F.10 for data on day 14). A notable non-significant trend was that 21.4% of the relaxation group reported that they thought they had low milk supply at day 21 (9/42), compared to 38.6% of the control group (17/43). Only 4 participants reported any direct breastfeeding on day 21 and this was one to two episodes in 24 hours.

**Table 6.10: Process indicators measured at day 21**

|                                                             | Relaxation Group<br>(n = 68) | Control Group<br>(n = 64) | Unadjusted effect estimate<br>(95% CI)   | Adjusted effect estimate <sup>34</sup><br>(95% CI) | p value |
|-------------------------------------------------------------|------------------------------|---------------------------|------------------------------------------|----------------------------------------------------|---------|
| <b>Skin to skin contact (hours)<sup>35</sup>, mean (SD)</b> | 2.2 (1.9)                    | 2.0 (1.8)                 | Median difference 0.0<br>(-1.2 to 1.2)   | Median difference 0.0<br>(-0.9 to 0.9)             | 1.000   |
| Median [IQR]                                                | 2 [1 to 3]                   | 2 [1 to 3]                |                                          |                                                    |         |
| Min to max                                                  | (0 to 8)                     | (0 to 9)                  |                                          |                                                    |         |
| Missing                                                     | 24                           | 23                        |                                          |                                                    |         |
| <b>Expressing episodes<sup>36</sup>, mean (SD)</b>          | 5.6 (2.4)                    | 5.9 (1.8)                 | MD -0.3<br>(-1.2 to 0.6)                 | MD -0.3<br>(-1.1 to 0.6)                           | 0.530   |
| Median [IQR]                                                | 6 [5 to 7]                   | 6 [5 to 7]                |                                          |                                                    |         |
| Min to max                                                  | (0 to 11)                    | (1 to 8)                  |                                          |                                                    |         |
| Solely breastfeeding                                        | 0 (0.0)                      | 0 (0.0)                   |                                          |                                                    |         |
| Not lactating                                               | 3 (6.7)                      | 0 (0.0)                   |                                          |                                                    |         |
| Missing                                                     | 20                           | 21                        |                                          |                                                    |         |
| <b>Expressing duration (minutes), mean (SD)</b>             | 151.5 (90.9)                 | 159.3 (84.9)              | Median difference 3.0<br>(-34.8 to 40.8) | Median difference -5.0<br>(-44.6 to 34.6)          | 0.802   |
| Median [IQR]                                                | 152 [105 to 200]             | 150 [110 to 195]          |                                          |                                                    |         |
| Min to max                                                  | (0 to 500)                   | (10 to 480)               |                                          |                                                    |         |
| Missing                                                     | 20                           | 21                        |                                          |                                                    |         |
| <b>Perception of low milk supply, n (%)</b>                 | 9 (21.4)                     | 17 (38.6)                 |                                          |                                                    |         |
| Missing                                                     | 26                           | 20                        |                                          |                                                    |         |
| <b>Direct breastfeeds<sup>37</sup>, mean (SD)</b>           | 0.1 (0.4)                    | 0.0 (0.2)                 |                                          |                                                    |         |
| Median [IQR]                                                | 0 [0 to 0]                   | 0 [0 to 0]                |                                          |                                                    |         |
| Min to max                                                  | (0 to 2)                     | (0 to 1)                  |                                          |                                                    |         |
| Missing                                                     | 40                           | 41                        |                                          |                                                    |         |

MD = mean difference. SD = standard deviation. IQR = interquartile range. CI = confidence intervals. Missing data are excluded from percentage denominators

<sup>34</sup>All are adjusted for multiple birth, gestation at birth and centre

<sup>35</sup>If two infants, then the sum of time spent in skin to skin contact with both

<sup>36</sup>An expressing episode is defined as where two breasts are expressed simultaneously or where two breasts are expressed sequentially, with the start time of the second breast expression within 10 minutes of the end time of the first breast expression or where one breast is expressed alone

<sup>37</sup>A direct breastfeed is defined as an episode at the breast where the mother felt the infant was sucking and swallowing some milk. If the infant is offered both breasts at a single episode this is one breastfeed. If the infant stops sucking or swallowing for 30 minutes or more, then any further feeding is a new breastfeed. If two infants, then the sum of all episodes with both infants

## **6.12 Dissemination of results to patients and the public**

Dissemination materials targeted at parents and the public are provided in Appendix G. These comprise an 'easy read' factsheet, a standard factsheet and a social media infographic. Chapter 4 described the involvement of trial participants in preparation of these resources.

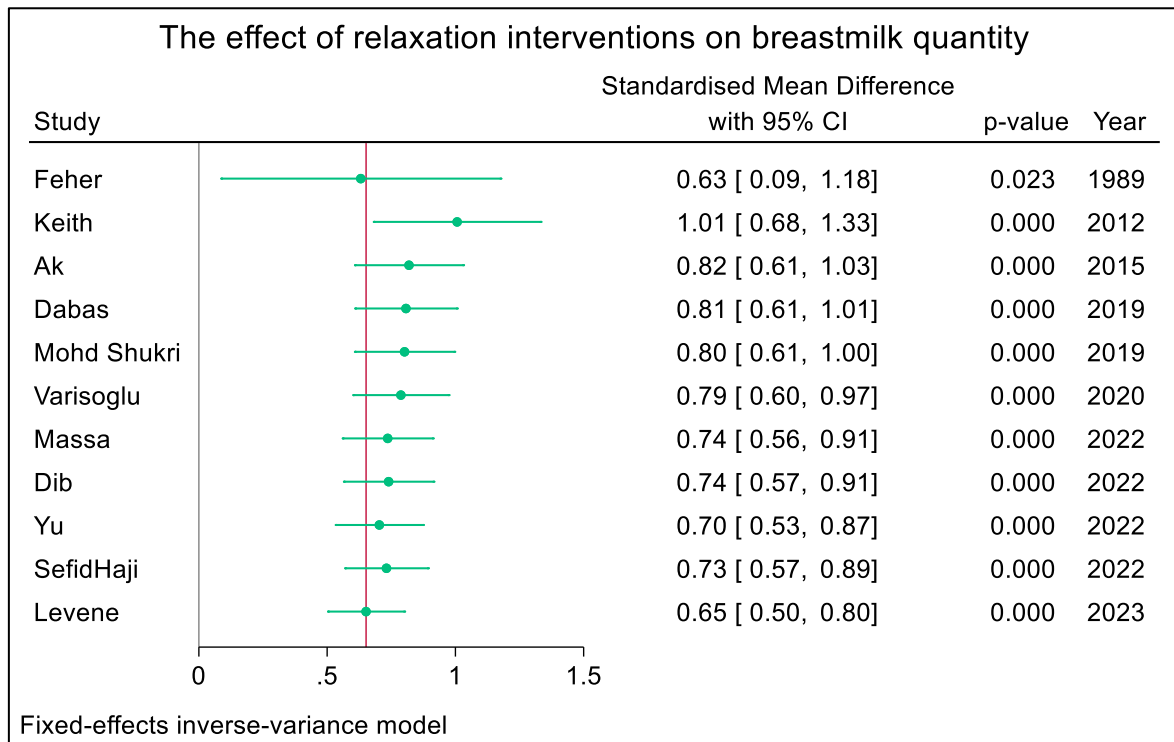
## **6.13 Inclusion of the study results in meta-analysis**

The adjusted primary outcome analysis was added to the meta-analysis of milk quantity presented in Chapter 3. Although the other included studies report unadjusted results, in my study the adjustment for measurement day is important to counteract the biased pattern of missing data so including an unadjusted analysis would be inappropriate.

The addition of my study to a fixed-effects meta-analysis reduced the overall standardised mean difference from 0.73 (95% CI 0.57 to 0.89, as reported in Figure 3.3) to 0.65 (95% CI 0.50 to 0.80, eleven studies). This is demonstrated in Figure 6.4, which shows the overall effect estimate as each consecutive study is added to the meta-analysis. My study was weighted at 15% within the final meta-analysis and was the second largest study included. Heterogeneity remained below the pre-specified threshold but increased from 29% to 47%. The funnel plot is provided in Appendix Figure F.2; addition of my study increases symmetry. Random effects analysis produced a similar result with slightly wider confidence intervals (SMD 0.64, 95% CI 0.41 to 0.87).

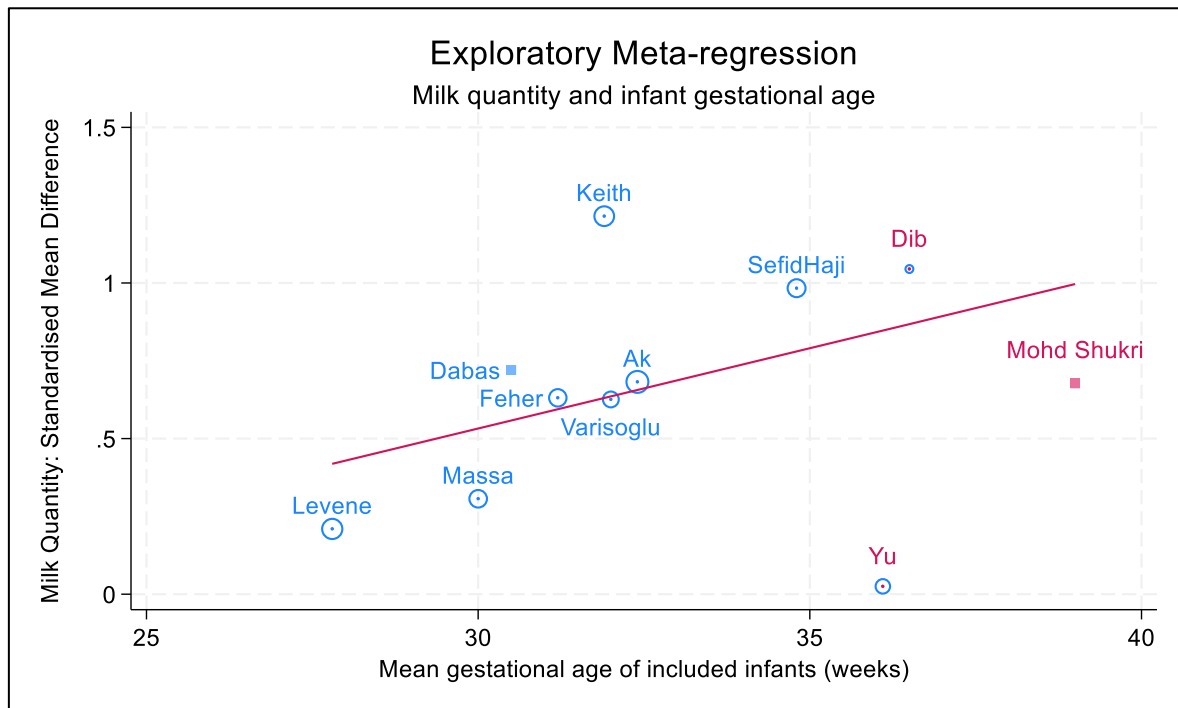


**Figure 6.4: Cumulative recursive meta-analysis of breastmilk quantity**



Post-hoc meta-regression did not show evidence for an association of the effect estimate with the mean gestational age of infants included in the individual studies (coefficient 0.05, 95% CI -0.08 to 0.18,  $p=0.38$ , nine studies). This is the regression line shown in Figure 6.5. Two studies did not report mean gestational age, which limits the already limited statistical power of this approach (meta-regression of less than ten studies is considered highly exploratory<sup>178</sup>). These are shown with an estimated mean gestational age in Figure 6.5 (derived from the eligibility range), but not included in the meta-regression analysis. The studies involving milk expression are shown with blue text labels and the studies involving breastfeeding are shown with pink text labels in Figure 6.5.

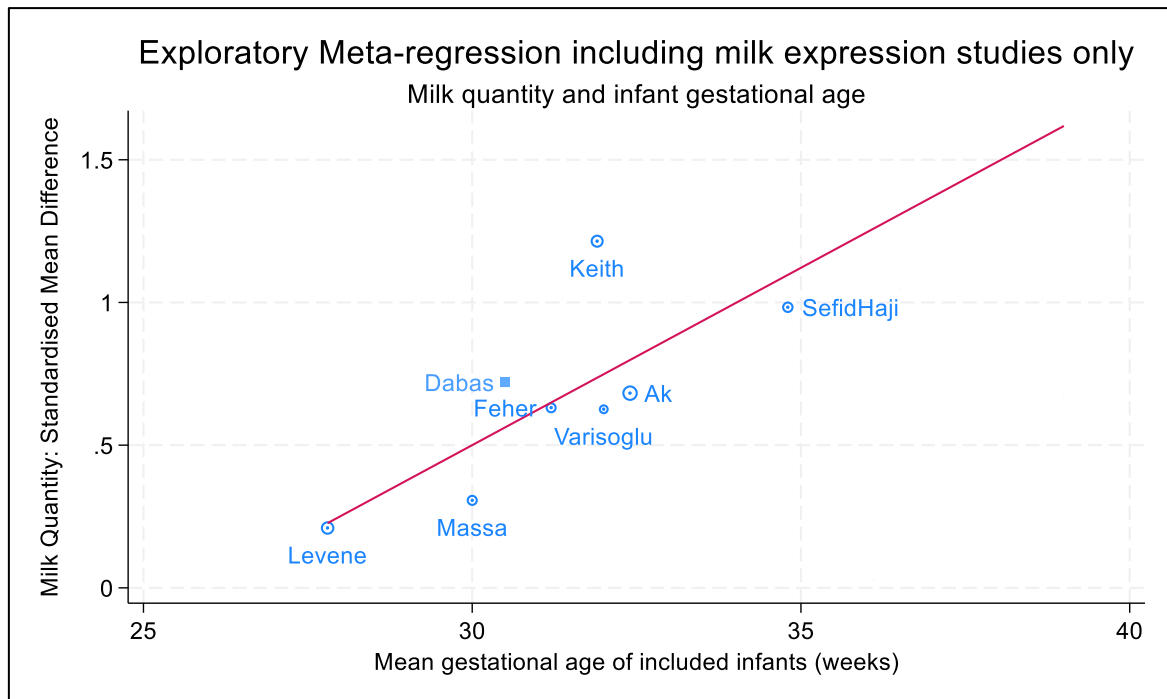
**Figure 6.5: Exploratory meta-regression of breastmilk quantity by infant gestational age**



Square data points show studies with estimated mean gestational age that do not contribute to the meta-regression line shown. Pink data labels (the three studies to the right of the graph) show studies of direct breastfeeding intake and blue data labels show studies of milk expression. The size of the surrounding bubble demonstrates the study weighting (a combination of the inverse of the standard error of the effect estimate and an estimate of between-study variability); a larger bubble represents higher weighting

Although the meta-regression did not detect a significant relationship, the size of the coefficient increased within the sensitivity analysis; when including only the studies of milk expression, the coefficient was 0.12 (95% CI 0.01 to 0.26,  $p=0.06$ , seven studies, Figure 6.6). In this model, gestational age explained 69% of between-study variance (adjusted  $R^2=69.2\%$ ).

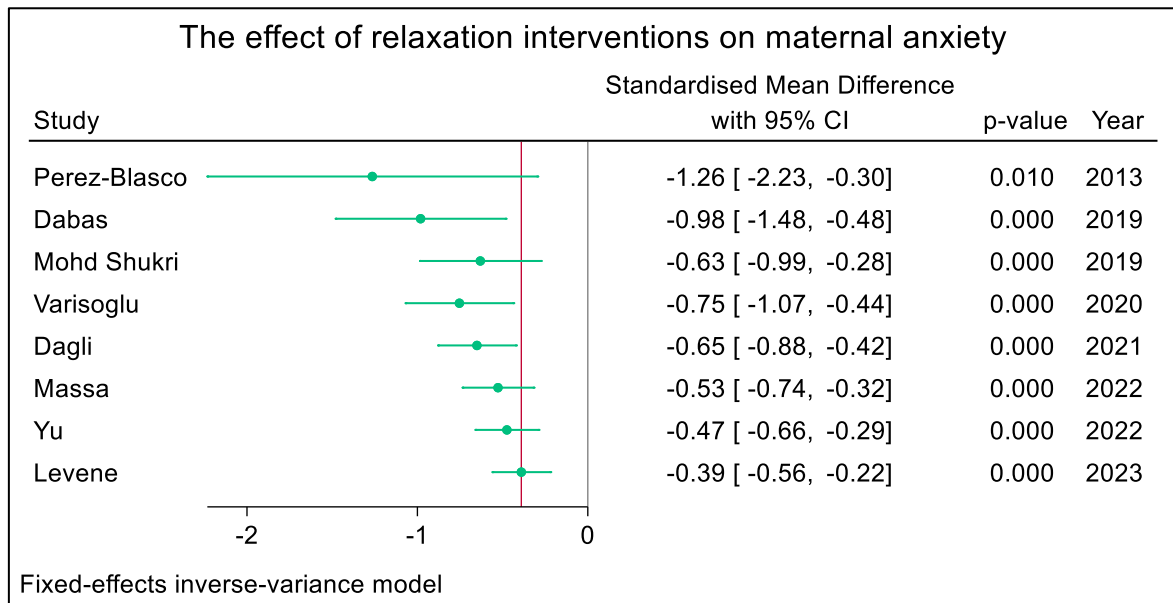
**Figure 6.6: Exploratory meta-regression of expressed breastmilk quantity by infant gestational age (sensitivity analysis)**



Square data points show studies with estimated mean gestational age that do not contribute to the meta-regression line shown. The size of the surrounding bubble demonstrates the study weighting (a combination of the inverse of the standard error of the effect estimate and an estimate of between-study variability); a larger bubble represents higher weighting

The secondary outcome of anxiety was also added to the relevant meta-analysis presented in Chapter 3. The addition of this study reduced the overall standardised mean difference from -0.47 (95% CI -0.66 to -0.29, as reported in Figure 3.14) to -0.39 (95% CI -0.56 to -0.22, Figure 6.7). My study was weighted at 16% in this meta-analysis. Heterogeneity remained high, increasing slightly from 60% to 66%. The funnel plot is provided in Appendix Figure F.2; adding my study increased symmetry.

**Figure 6.7: Cumulative recursive meta-analysis of anxiety**



It is notable that for both these outcomes, our study continues the existing trend to a decrease in the effect estimate size. This may be explained by increasing study quality over time as interest in this area has grown, or involvement of populations at higher risk of low milk quantity and anxiety. Comparing recruitment and publication dates did not show evidence of increased publication time lag for studies with negative or smaller effect estimates compared to those with more positive results so time lag bias is an unlikely cause of this pattern.

The study outcomes of any and exclusive breastmilk were added to the much smaller individual meta-analyses for these two outcomes, with no significant change to their conclusions. The pooled risk ratio for exclusive breastmilk at around two months of age was 0.98 (95% CI 0.87 to 1.09, three studies). For any breastmilk this was 1.07 (95% CI 0.94 to 1.22, two studies).

## 6.14 Discussion

### 6.14.1 The effect of relaxation on the studied outcomes

The trial showed no evidence of an effect of a guided relaxation and lactation-specific visualisation recording on any lactation outcomes, anxiety or post-traumatic stress, despite high adherence. The majority of participants who provided data, liked the intervention and found it relaxing.

This study, like two others<sup>164,172</sup>, was powered with reference to a NICU study<sup>203</sup> showing a dramatic increase in milk yield (more than 500ml) with a very similar intervention recording. However the two more recent studies showed a smaller non-significant positive effect estimate (132g<sup>164</sup> and 145g<sup>172</sup>), which is more similar to this report. Meta-analysis demonstrates a gradual reduction in the effect estimate size as more studies are added, including this one. However, a moderate positive effect on milk quantity remains the conclusion of the most up to date meta-analysis and the reduction in effect estimate with new studies is small.

This study recruited participants with a higher level of prematurity than any other study in this area. Infants of the lowest gestation generally have worse breastmilk feeding outcomes than those with more moderate prematurity<sup>91,135,136,138,140,144,147,255,256</sup>, and there is a suggestion (but no concrete evidence) of a positive association of infant gestation and effect size both in sub-group analysis of this study and in meta-regression. Both are underpowered to identify if this is a true relationship so this is a hypothesis that would require further assessment.

Although meta-regression was not a pre-specified analysis, the choice of gestation as a pre-specified subgroup for meta-analysis and as the single RCT sub-group analysis to be tested with statistical inference demonstrates its a priori identification as potentially important to the effect of relaxation. It is considered very difficult to demonstrate significant relationships with less than ten studies included in meta-regression<sup>178</sup>, so the finding of a trend nearing statistical significance is notable.

The mechanism by which lower gestational age at birth might impair the effect of relaxation is likely the level of physiological lactation challenge experienced; for example if the maturation of mammary tissue is less advanced, there may be less capacity to respond to lactogenic hormones. Gestation is not a 'pure' marker of lactation challenge. For example, late preterm infants may have worse lactation outcomes than infants with a higher level of prematurity, although that may relate to poorer feeding support rather than underlying physiology<sup>33,257</sup>. This may explain why the meta-

regression result was strengthened when looking only at studies involving milk expression. Our results therefore suggest a new hypothesis that the higher lactation challenge experienced by our population may be a factor in the smaller effect estimate reported here compared to other studies<sup>167,202,203,200</sup>.

An increase in milk quantity of 74g, as observed in this trial, would have clinical importance in this setting; similar to that of the galactagogue domperidone (94g in meta-analysis<sup>259</sup>). Even small increases in milk quantity provided to very preterm infants have potential impact. For example, each 10ml/kg/day extra breastmilk is associated with a decrease in rehospitalisation risk by 30 months of age of 5%<sup>7</sup> and with \$5340 reduction in hospital costs<sup>9</sup>.

Participants with higher adherence with the intervention had a higher milk quantity than those with lower adherence. Higher adherence was not associated with any other variables that might suggest this was due to confounding (although this cannot be excluded). This increased effect in more adherent participants has been demonstrated in several other trials<sup>162,164,172</sup>, including one which adjusted for associated confounders<sup>164</sup>. It is therefore possible that increased adherence is associated with increased effect on milk quantity, although this suggestion should be viewed cautiously. In contrast, perception of relaxation was not associated with milk quantity. This is in line with other studies that have shown a decoupling of relaxation or stress perception, with physiological or biochemical changes associated with a relaxation response<sup>108,163</sup>.

The impact of relaxation on maternal anxiety is variable in previous RCTs, with three showing a reduction<sup>169,174,200</sup> and three showing no difference<sup>162,164,172</sup>. There was a high level of anxiety in our population, with mean STAI at baseline of 52 in comparison to 31 in a previous NICU study that demonstrated significant reduction in anxiety with a relaxation intervention<sup>200</sup>. A further NICU study<sup>164</sup> with an even higher level of participant anxiety (mean control group STAI of 71) also showed no evidence of effect from relaxation. This may suggest that relaxation interventions are less effective in reducing anxiety when baseline anxiety is very high. The level of anxiety seen in this

study was higher than in a general population of mothers with infants admitted to NICU<sup>67</sup>, but this is likely due to the extreme level of prematurity in this cohort.

No previous studies have reported on post-traumatic stress reactions after use of a lactation-focused relaxation/visualisation. There was no statistically significant change in post-traumatic stress reactions using the PCL-5. However some participants subjectively related use of the recording to reframing their experience of birth trauma and there was a trend to fewer relaxation group participants exceeding the threshold of clinically significant post-traumatic stress. The prevalence of clinically important PTS reactions in this sample was as expected for high risk mothers in the post-partum period<sup>260</sup>. Further study of the impact of relaxation on post-traumatic stress is merited, given the paucity of interventional research on this important outcome and the high burden of post-traumatic stress seen in NICU parents<sup>67</sup>.

This study provides no evidence that relaxation changes the rate of any or exclusive breastmilk provision, which is in accordance with the rest of the limited literature on these outcomes<sup>162,164,198</sup>.

When considering possible harm of the intervention, relaxation group participant comments suggested that their experience was affected by the lack of variety in the relaxation material, and some would have preferred calming music instead of, or combined with, the voice recording. The increased loss to follow-up in the intervention group and the reduced number of multiparous women choosing to take part suggests that the intervention might have been burdensome to some. This concentration of primiparous participants has been seen in another UK study of relaxation<sup>163</sup>. In addition, at least 12% of the intervention group disliked the intervention. Comments showed that some found it a chore or felt pressure to listen. In addition to being unpleasant for participants, these factors may have impaired adherence and/or effectiveness. However, perception of relaxation and physiological signs of a relaxation response are not necessarily closely linked<sup>108</sup>.

Although the qualitative comments were generally brief and only received from a selection of participants, they provided valuable insight on the participants' experiences. Some comments

echoed those reported in a previous publication, which reported that the use of mindfulness helped NICU parents focus on connection with the infant<sup>206</sup>.

### **6.14.2 Strengths and Limitations**

One strength of this study was the close attention paid to identifying potential bias and minimising its impact on the study conclusions. Therefore, the key limitations, which are the level and unequal pattern of missing data, have been predominantly mitigated and the study has a lower risk of bias than many others reviewed in Chapter 3<sup>167,174,175,202,200</sup>. There was a large reduction in the primary outcome effect estimate after adjustment, and when using sensitivity analyses designed to address potential bias from study design and missing data. This demonstrates the need for this approach.

A further strength of the study is its external validity. The study was multi-centre, including four neonatal units with a range of background breastmilk feeding prevalence and support structures. Participants were diverse in socioeconomic and ethnic background. The spread of gestational age at birth was also wide, including many participants who had experienced the most extreme level of prematurity.

A limitation of the study was the unblinded nature, which could have led to the relatively high level of relaxation use in the control group. However, the background rate of relaxation practice during standard care is unknown in this setting, so it is difficult to assess if this was contamination due to the trial context (i.e. because of the information provided during recruitment) or not. However, 53% of women of child-bearing age in a trial of relaxation interventions reported regularly using a form of relaxation prior to recruitment<sup>261</sup>. This suggests that the rate of relaxation reported in the control group here is more likely to reflect the expected background rate, rather than contamination related to the trial consent process or interaction with individuals in the relaxation group. Most RCTs in this field do not attempt to identify contamination.



It was notable that the intervention group increased their use of non-intervention relaxation material in addition to a high level of adherence with the intervention – this enhancement of the frequency of relaxation experiences in the intervention group has also been seen in another trial<sup>163</sup>. It may reflect an increased interest in relaxation, or an attempt to replace the intervention with alternative methods of relaxation that the participant prefers.

### **6.14.3 Risk of bias**

There was no evidence that participant reported outcomes were influenced by a belief in the intervention or desire to please the research team, which is a possibility in unblinded trials. Mental health scores would be most vulnerable to this possibility due to their self-reported nature and showed no signal of treatment effect. There was also no signal of difference in expressing behaviours such as duration or frequency of expressing.

The study was relatively small and therefore demonstrated some imbalance in baseline variables. Control group participants had slightly lower levels of education, socioeconomic advantage, caesarean birth and birth in the recruiting centre; and shorter duration of prior breastmilk feeding. These variables (apart from reduced caesarean birth) are associated with worse breastmilk feeding outcomes<sup>150–154</sup> which would have the potential to artificially inflate the intervention effect estimates. The exploratory results described in Chapter 7 will note that prior breastfeeding experience of more than six months was particularly highly associated with expressed milk yield. Therefore the fact that 25% of the intervention group fell into this category (15/60), compared to 19% of the control group (11/58), may have had particular impact to artificially inflate the effect estimate.

If assessed with the same criteria used in Chapter 3, the risk of bias for the trial outcomes reported here would be low for the primary outcome and two of the secondary outcomes, as shown in Table 6.11. There would be some concern for risk of bias in domain three (missing data) for the mental health outcomes, the rate of milk expression (measured at day 21) and the four months' CA feeding

assessment. Domain four (measurement) would additionally be classified as some concerns for mental health assessment due to the subjective nature of self-report in an unblinded trial.

**Table 6.11: Risk of Bias assessment for the randomised trial**

| Outcome                     | D1  | D2  | D3           | D4           | D5  | Overall      |
|-----------------------------|-----|-----|--------------|--------------|-----|--------------|
| 24-hour milk quantity       | Low | Low | Low          | Low          | Low | Low          |
| Expressing $\geq 750$ g     | Low | Low | Low          | Low          | Low | Low          |
| Milk expression rate        | Low | Low | Some concern | Low          | Low | Some concern |
| Anxiety score (STAI)        | Low | Low | Some concern | Some concern | Low | Some concern |
| PTS score (PCL-5)           | Low | Low | Some concern | Some concern | Low | Some concern |
| Feeding status 36 wks' PMA  | Low | Low | Low          | Low          | Low | Low          |
| Feeding status 4 months' CA | Low | Low | Some concern | Low          | Low | Some concern |

D1 = Domain 1 (randomisation); D2 = Domain 2 (deviations from intended intervention); D3 = Domain 3 (missing data); D4 = Domain 4 (outcome measurement); D5 = Domain 5 (result selection). STAI = Spielberger State Trait Anxiety Index. PTS = post-traumatic stress. PCL-5 = Post-traumatic stress Checklist for DSM-5. PMA = post-menstrual age. CA = corrected age.

## 6.15 Conclusions

This RCT did not show evidence of an improvement in lactation or mental health outcomes with provision of relaxation. The trial was powered to detect an increase of 155g because of a prior study showing a much larger effect of relaxation; the lower effect estimate reported here (74g) would also have clinical impact in this setting of very preterm birth and high lactation challenge.

Participants predominantly enjoyed the relaxation intervention and perceived it to be relaxing; qualitative data showed that many participants valued the recording. Minor harm to participants was noted, as some disliked the recording and found it burdensome.

## 6.16 Summary

This chapter has presented the results of the randomised controlled trial undertaken for the thesis, and discussed their initial implications. Chapter 8 will integrate the trial findings reported here with the systematic review findings and discuss how these should be interpreted to form a clinical recommendation for families on the use of relaxation. The next chapter, Chapter 7 will pose an exploratory question of the relationship of the pattern of expressing with lactation outcomes, in order to inform clinical recommendations to families about how to optimally express.

# **Chapter 7: Exploratory analysis of the dynamics of milk expression**

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## **7.1 Chapter Overview**

The previous chapter presented the results of the randomised component of the randomised controlled trial, and their initial implications. This chapter will present the results of a pre-specified exploratory analysis using this cohort of data. First the chapter will introduce the rationale for the exploratory questions, the existing evidence in this area and the methods applied to the analysis. The initial implications of the results will also be discussed.

## **7.2 Introduction**

Chapter 4 demonstrated that mothers have a large number of questions about the optimal dynamics of expressing. The dominant specific questions on optimal expressing were how frequently to express and the recommended pattern of expressing sessions. The pattern of expressing relates to the length of each session, the gap between sessions and the timing of sessions across the 24-hour period (circadian pattern). The question of the importance of expressing at night was specifically raised and mothers commented on the negative psychological and physical impact of night expressing.

Therefore, the thesis objectives included undertaking observational analysis of the data collected as part of the randomised controlled trial to explore the relationship between expressing patterns and lactation outcomes, and to use these insights to suggest any changes required in clinical recommendations.

## **7.3 Relevant existing evidence**

### **7.3.1 Direct breastfeeding patterns**

A relatively small number of papers have been published describing the breastfeeding patterns of healthy infants who are directly breastfeeding. Some of this literature was reviewed in 2007, although not

systematically<sup>131</sup>. This review suggests that the physiological norm is for infants to breastfeed frequently through the first weeks and months after birth. In a Japanese study of 140 healthy, term infants breastfeeding responsively, feed frequency was  $7.4 \pm 3.9$  times in the second 24 hours after birth<sup>262</sup>. A range of studies have identified 7 to 10 times per day as an average breastfeeding frequency between the ages of two weeks and three months in the UK, Australia and USA<sup>132,263,264</sup>. The gap between feeds was on average 3 hours  $\pm$  41 minutes<sup>263</sup>, with a range of 40 minutes to 11 hours.

In a UK study of 91 mothers, the mean total duration of time at the breast was 217.5 minutes at day seven after birth and 202.8 minutes at four weeks<sup>132</sup>. In a USA study it was  $162 \pm 50$  minutes during the first two weeks and  $158 \pm 63$  minutes at one month<sup>264</sup>. An Australian study showed that when breastfed infants are feeding at night, these are normally the highest volume feeds in the 24-hour period but happen at lower frequency compared to daytime hours. When breastfed infants are not feeding at night, the highest volume feeds are in the morning after the long night-time gap<sup>263</sup>.

Milk is synthesised continuously in the breast so the longer the gap between feeds, the more milk is theoretically available for extraction. As expected, the volume of milk that the infant drinks at any given feed is therefore partly dependent on the time since last feed, although infants rarely drink all of the milk available<sup>265,266</sup>. However, inhibitory feedback occurs if there is milk present in the breast for a prolonged period without being drained, and by contrast when the breast has been well-drained, the rate of milk synthesis appears to increase<sup>266,267</sup>. This is the theoretical reason that frequent feeding/expression is thought to be important to increase or maintain overall milk production.

It is important to note that there is a large individual variability in the amount of milk that can be present in the breast before activation of inhibitory feedback ('storage capacity') – meaning that different individuals have different capacity to tolerate infrequent breast drainage<sup>266,267</sup>. This suggests that some individuals might need to breastfeed or express milk more frequently than others in order to achieve the same yield. Storage capacity can vary over time and between pregnancies<sup>131</sup>.

Evidence on the relationship of breastfeeding frequency and 24-hour milk intake during direct breastfeeding is conflicting. A correlation has been shown in one Japanese study in the first 24 hours after birth<sup>262</sup> ( $r=0.5$ ) and in two Australian studies spanning one to 15 months after birth<sup>268,269</sup> ( $81.1\text{g} \pm 18.5$  increased 24-hour intake per extra feed<sup>269</sup> and  $r=0.4^{268}$ ), but not in three studies between two weeks and six months of age in Australia and USA<sup>263,264,270</sup>.

### **7.3.2 Methods of evidence review on expressing patterns**

A systematic search was performed to explore the evidence related to expressing patterns and lactation outcomes in a neonatal unit setting, with the final search taking place on 8<sup>th</sup> October 2023. The search structure and flowchart are provided in Appendix Table H.11 and a detailed description of included studies is presented in Appendix Table H.12. One RCT and 24 observational reports were identified contributing data related to this area, including 1898 participants in total. Individual studies reported on between 16 and 415 participants, taking place predominantly in the USA<sup>31,39,41,43,44,76,91,134,153,253,271–275</sup> but also in Israel<sup>276</sup>, Canada<sup>42</sup>, Japan<sup>136,277</sup>, Finland<sup>33</sup>, Australia<sup>40,62</sup>, China<sup>36,278</sup> and the UK<sup>38</sup>. Most studies included very low birthweight infants and/or infants born at less than 32 or 34 weeks' PMA. Three studies<sup>33,272,278</sup> included all preterm infants or all low birth weight infants in a NICU setting.

### **7.3.3 Evidence related to expressing patterns**

The one RCT identified has significant shortcomings<sup>272</sup>. Mothers of preterm infants who were not breastfeeding were randomised to a recommendation to express three or fewer times a day (in practice  $2.4 \pm 0.6$  times/day), or four or more times a day (in practice  $5.7 \pm 0.6$  times/day). They were randomised for the period between day five and 11 after birth and then re-randomised to the same two potential arms for the period between day 11 and 18, with no washout period. Although 25 mothers were randomised, not all completed both stages and the results are difficult to interpret because some participants experienced both periods of the crossover design and some did not. In paired analysis, there was a significant increase in milk yield with more frequent expressing for participants who experienced

both allocations ( $342 \pm 229$  mL in the high frequency period and  $221 \pm 141$  mL in the low frequency period). When looking at participants who only provided data for their first expression week as a parallel group comparison, there was no difference between the two allocations ( $177 \pm 96$  mL in the high frequency group and  $123 \pm 118$  mL in the low frequency group). Both of these sub-groups were extremely small ( $n = 9$  and  $n = 7$ ).

The 24 observational studies used highly variable definitions of expressing frequency and long-term lactation success, making evidence synthesis difficult. The majority of the studies support the association of expressing frequency and contemporaneous yield measured at between day one and week ten after birth<sup>36,38–41,43,62,76,253,273,274,276</sup> (i.e. the expressing frequency and the milk yield were measured or averaged over the same time period). Six reported conflicting results at different contemporaneous timepoints assessed within the same study, with no consistent patterns<sup>44,91,134,271,275–277,279</sup>. Five studies with 323 participants showed no association of expressing frequency and contemporaneous milk yield in the first four days after birth<sup>277</sup>, at day seven<sup>276</sup>, week two<sup>33,44</sup>, week three<sup>42</sup> and week four<sup>44</sup>.

Six studies with 386 participants reported weak to moderate correlation coefficients for contemporaneous measurement of expressing frequency and milk yield, ranging from 0.32 to 0.51<sup>38,39,43,76,254,275</sup>. Three studies with 81 participants reported increased adjusted odds of higher contemporaneous milk yield<sup>43,274,277</sup> and increased adjusted milk yield<sup>41</sup> for each increment of expressing frequency (one further study showed increased odds of higher milk yield, but this was no longer significant after adjustment<sup>277</sup>).

Three studies with 131 participants reported that “high frequency” expressing (defined as at least 6<sup>36,39,40</sup> or 7<sup>273</sup> times per day) compared to “low frequency” expressing (less than 4<sup>40</sup>, 6<sup>36,39</sup> or 7<sup>273</sup> times per day) was associated with significantly increased contemporaneous milk yield in the first month after birth. Mean differences were 220 mL (41.7 to 398.3)<sup>273</sup>, 223 g (77 to 370)<sup>40</sup> and 313 mL (119.1 to 506.9) per day<sup>39</sup>. An additional study with 30 participants reported that on average, high frequency expressing (at

least six times per day, compared to less than six per day) during the first 42 days was associated with 621.2 mL (278.8 to 963.6) increase in milk yield at 42 days<sup>36</sup>.

Expressing frequency between day 7 and 21<sup>31,134,153</sup> was associated with longer term (non-contemporaneous) outcomes in three studies with 629 participants; for example, increased adjusted odds of any breastmilk at discharge of 7.5 (3.3 – 17.1) for each increment in daily expressing frequency in the first two weeks after birth<sup>153</sup>. However three studies with 185 participants did not show an association between early expressing frequency and later lactation outcome<sup>38,276,277</sup>.

One small USA study (n = 32) analysed the maximal gap between expressions in a 24-hour period. It found no association with daily milk yield at either two or four weeks after birth<sup>44</sup>. This was also the only study to look at the duration of expressing, finding no relationship between total daily expressing duration and contemporaneous milk yield at either two or four weeks after birth<sup>44</sup>.

Finally, no reports were identified that explored the association of circadian timing of expressing sessions and lactation outcomes, in either the short or long term. In clinical practice mothers are often told that night-time expression is required not only to prevent a long gap between sessions but also because prolactin levels are highest at night. There is a nocturnal rise in basal prolactin in non-lactating and lactating individuals (nocturnal being defined as 2400-0600 in one study<sup>280</sup> and 2300-0700 in another<sup>281</sup>). However this may be related to the physiological state of sleeping<sup>280</sup> and occurs despite less frequent night-time breastfeeding episodes and sometimes no breastfeeding episodes at all in the healthy mother/infant pairs studied<sup>280,281</sup>.

Some authors hypothesise that the night-time rise in basal prolactin is a mechanism to protect milk supply from the longer gaps between breastfeeding episodes<sup>281</sup>. Rather than supporting the hypothesis that night-time expression is inherently important because of circadian prolactin patterns, this would actually imply that longer gaps between expressions can be tolerated better at night-time than during the day. However, night-time expressing may be required in order to fit a certain frequency of expressing into the 24-hour period, or to prevent a maximal gap of a specific length, if these are shown to be important.

### **7.3.4 Summary of evidence review**

When taken together, the literature on expressing pattern predominantly supports an association of higher frequency expressing with increased contemporaneous milk yield. No specific threshold has strong evidence as a clinical recommendation but several studies support expressing at least 6 or 7 times per day. Total time spent expressing and longest gap between expressions have not been shown to be associated with milk yield in the single study reporting these. No studies were identified that specifically looked at the circadian timing of expressing sessions.

Key questions for exploratory analysis are therefore:

1. Is expressing frequency associated with contemporaneous 24-hour milk yield and/or long-term breastmilk feeding outcomes? Does any association change with increasing time from birth?
2. Is the gap between expressions, and the longest gap in the 24-hour period, associated with contemporaneous milk yield and/or long-term breastmilk feeding outcome? Does any association change with increasing time from birth?
3. Are current UK clinical recommendations for expressing frequency (at least eight times per day) and longest gap (less than 6 hours) associated with good lactation outcome?
4. Is there an independent association of circadian timing of expressing sessions with milk yield once the gap between expressing sessions is adjusted for?

## **7.4 Methods**

### **7.4.1 Variables included**

The exploratory analysis uses data collected for the RCT, whose methods are described in Chapter 5. Three types of outcome variables were used for exploratory analysis; 24-hour expressed milk yield, exclusive breastmilk at 36 weeks' PMA (both also RCT outcomes) and expressing-session level milk yield.

The key explanatory variables of expressing pattern that are examined in this analysis are expressing frequency, the gap between expressions and the duration of expressing. There were some complexities



in deriving and defining these variables from the expressing logs. Where a participant used more than one expressing bottle per breast in one expressing session, there was a variety of ways they recorded this, which meant that multiple recorded logs could pertain to a single session. Two or more logs were considered to pertain to a single expressing session if fulfilling any one of the following three conditions; logs had identical start times; the start of one log was within the expressing period of another log; or there was a gap of less than 10 minutes between the end of one log and the start of another log.

The gap between expressing sessions was a derived variable (the number of hours from the start time of one expressing session to the start time of the prior session). For the first logged session of the measurement day (where the timing of the previous session was unknown), the final logged session of the same 24-hour period was used as a proxy for the previous session. For example, if a participant logged an expressing session at 9am, 1pm and 9pm on day four, the gap to prior session was assigned as 12 hours for the first session (assuming that the participant expressed at 9pm on day 3 because of the evidence that they expressed at 9pm on day four). The gap to prior session for the other two logged sessions would be calculated from the data provided as 8 hours for each. This method means that if only one session was logged on a measurement day, the gap to prior session was allocated as 24 hours. Sensitivity analysis was performed to assess the impact of this assumption.

A further derived variable was classification of delayed secretory activation (lactogenesis II, or milk 'coming in'). Secretory activation is the onset of copious milk production and is classified as delayed if it has not occurred by day 4 after birth<sup>282</sup>. Participants who had documented expressing at least 20g of milk for two consecutive sessions at day 4 were considered to have achieved secretory activation. For mothers who have given birth very prematurely, this may be the most accurate definition because they more rarely have a feeling of breast fullness or biochemical changes within the milk<sup>253</sup>. As a pragmatic measure where only one expressing session was logged, lactogenesis II was assumed to have been achieved if the total quantity was more than 40g.

Confounding and explanatory variables considered for inclusion in this analysis comprised expressing-related and sociodemographic variables (taken from the baseline questionnaire). Expressing-related confounders were time to first expression after birth (asked at recruitment), expressing mode (for example, hand or electric pump), simultaneous expression and skin to skin contact duration (all recorded on expressing logs and associated questionnaires). The sociodemographic variables available are those listed in section 6.3 above.

#### **7.4.2 Statistical Analysis**

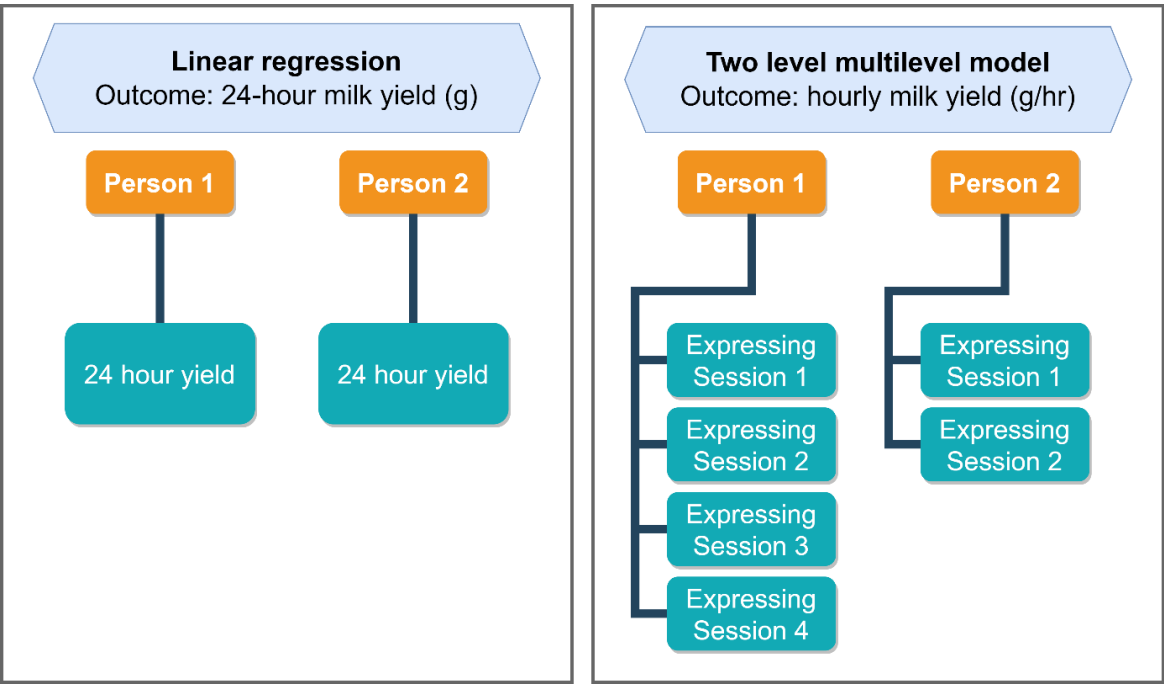
An exploratory statistical analysis plan was prepared prior to data lock, provided in Appendix H. This covers a broader range of questions than those considered in this chapter; further analyses relating to the time to first expression and the relationship of milk quantity with long term breastmilk feeding will be published elsewhere due to space constraints within the thesis.

Stata 18.0 was used for analysis. Descriptive analysis used mean and standard deviation or median and interquartile range as appropriate. Correlations were assessed using Pearson coefficient (referred to as “*r*”). Chi squared test was used for categorical data where appropriate. The major outcome of the analysis (24-hour expressed milk yield as a continuous variable) was analysed using linear regression for individual measurement days separately, as demonstrated in Figure 7.1.

Where multiple timepoints for explanatory variables were available, only contemporaneous measurements were included. For example, when the outcome was milk quantity on day four after birth, a variable such as expressing frequency would only be included in the regression model when measured at day four, not when measured at day 14 or day 21. Consideration was made of using multilevel modelling to analyse repeated measures of 24-hour milk yield on the multiple measurement days, with up to three measurement days per participant. This analysis was judged too complex for the sample size because of evidence of multiple interactions between key explanatory variables and measurement day.

Exclusive breastmilk at 36 weeks' PMA (a binary variable) was analysed using Poisson regression with robust standard errors. Milk yield at the individual expressing-session level (a continuous variable) was also analysed for individual measurement days to examine the association of expressing session-level explanatory variables such as session timing. Here, repeated measures of expressing sessions on an individual measurement day were analysed using multilevel modelling, with individual as a random effect, as demonstrated in Figure 7.1. At the expressing session level, the specific outcome is hourly milk yield (session yield in grams, divided by the gap between the current and previous session in hours, resulting in yield per hour). This has been used in prior work<sup>40</sup> and is useful because it accounts for the competing factors of individual session yield and number of sessions per day that both contribute to 24-hour yield.

**Figure 7.1: Representation of linear regression and multilevel modelling used in this analysis. In both cases each individual measurement day is analysed separately at day 4, 14 and 21**



For the analysis of 24-hour milk yield, the key expressing dynamic variables (expressing frequency, longest gap between expressions and duration of expression) were fitted in a multivariable model. Where close correlation was seen between these variables, only one was included, to avoid collinearity.

Adjustment was made for potential confounders related both to baseline sociodemographic and perinatal factors, and to expressing-related factors such as time to first expression, the type of expression used and skin to skin contact duration. These potential confounders were identified from the literature. These variables were included as candidates for multivariable analysis if they showed association with the outcome in univariable analysis with  $p < 0.2$  and retained in the final model if  $p < 0.05$  after stepwise removal. However, the following variables were retained in the model if  $p < 0.2$  because of their particular theoretical importance in the literature and with recognition of the small sample size: maternal age, maternal education, breastfeeding intention and multiple birth.

Three forms of post-hoc sensitivity analyses were performed to assess potential bias. Firstly, removing any major outliers for 24 hour milk yield, secondly removing participants who did not submit expressing logs on all three measurement days (day 4, 14 and 21), and thirdly removing the derivation assumption related to assigning a gap between expressing sessions to the first logged session of each day as described above.

## **7.5 Results**

### **7.5.1 Expressing characteristics**

556 expressing sessions were logged by 103 individuals at the day 4 assessment, 577 by 91 individuals at day 14 and 560 by 91 individuals at day 21. 108 individuals contributed data for at least one timepoint. Table 7.1 shows the expressing characteristics of the cohort at both the 24-hour level (based on data from 87-103 individuals) and the session level (based on data from 471 to 574 sessions). The median time to first expression attempt after birth was 6 hours (interquartile range 3 to 12).

Participants were predominantly using an electric pump at all sessions (75.0% at day 4 and 87.4% at day 21) and expressing both breasts simultaneously at all sessions (79.4% on day 4 and 83.9% on day 21). The majority of individuals expressed at least once in the night-time period (2300 to 0700); 75.7% (78/103) on

day 4 and 83.9% (73/87) on day 21. On day 4, 10.7% of participants expressed only once a day (11/103), whereas on day 14 and 21 this was 3.3% and 4.4% (3/91 and 4/91 respectively).

**Table 7.1: Expressing related variables over time**

|                                                         | Day 4            |                       |          | Day 14           |                      |          | Day 21          |                        |          |
|---------------------------------------------------------|------------------|-----------------------|----------|------------------|----------------------|----------|-----------------|------------------------|----------|
|                                                         | Mean<br>(SD)     | Median<br>(IQR)       | <i>n</i> | Mean<br>(SD)     | Median<br>(IQR)      | <i>n</i> | Mean<br>(SD)    | Median<br>(IQR)        | <i>n</i> |
| <b>Individual level characteristics</b>                 |                  |                       |          |                  |                      |          |                 |                        |          |
| <b>Milk yield<br/>(g)</b>                               | 221.0<br>(208.3) | 154.7<br>(66.2–295.7) | 101      | 490.6<br>(345.6) | 454.1<br>(236.7–653) | 90       | 560<br>(386.4)  | 490.2<br>(247.5–830.2) | 90       |
| <b>Expressing<br/>episodes</b>                          | 4.9<br>(2.2)     | 5<br>(4–7)            | 103      | 6.0<br>(1.9)     | 6<br>(5–7)           | 91       | 5.7<br>(2.1)    | 6<br>(5–7)             | 91       |
| <b>Expressing<br/>duration<br/>(mins)</b>               | 132<br>(84.7)    | 115<br>(80–178)       | 103      | 161.5<br>(78.6)  | 160<br>(120–204)     | 91       | 155.2<br>(87.7) | 150<br>(110–200)       | 91       |
| <b>Longest<br/>expressing<br/>gap<sup>38</sup>(hrs)</b> | 9.9<br>(5.9)     | 8<br>(6–12)           | 102      | 7.4<br>(4.1)     | 6<br>(5–8.5)         | 89       | 7.5<br>(4.2)    | 6.5<br>(5–8.5)         | 87       |
| <b>Session level characteristics</b>                    |                  |                       |          |                  |                      |          |                 |                        |          |
| <b>Duration<br/>(mins)</b>                              | 27.6<br>(13.7)   | 25<br>(18.5–30)       | 556      | 27.1<br>(10.9)   | 26.5<br>(20–30)      | 574      | 27.4<br>(11.8)  | 25<br>(20–30)          | 560      |
| <b>Gap to<br/>prior<br/>session<br/>(hrs)</b>           | 4.9<br>(4.1)     | 3.7<br>(2.8–5.3)      | 518      | 4.1<br>(2.9)     | 3.5<br>(2.8–4.4)     | 523      | 4.2<br>(2.8)    | 3.4<br>(2.8–4.6)       | 501      |
| <b>Milk yield<br/>(g)</b>                               | 44.5<br>(45.3)   | 31.3<br>(15.8–56.2)   | 501      | 82.2<br>(62.3)   | 71<br>(36.6–112.7)   | 537      | 97.2<br>(66.4)  | 86<br>(47.4–139.4)     | 519      |
| <b>Hourly<br/>milk yield<br/>(g/hr)<sup>39</sup></b>    | 11.5<br>(12.4)   | 8.0<br>(3.3–15.0)     | 471      | 23.1<br>(18.9)   | 19.4<br>(9.8–32.4)   | 495      | 27.6<br>(21.2)  | 23.6<br>(13.2–38.5)    | 479      |

*SD = standard deviation. IQR = interquartile range*

On day 4, 47.9% participants reported any skin-to-skin contact with their infant/s (46/96), whereas on day 21 this was 77.7% (66/85). Median duration of skin-to-skin contact was 0 hours on day 4 and 2 hours on day 21. There were minimal direct breastfeeding attempts at any timepoint.

Few participants achieved UK recommendations on optimal expressing set by UNICEF BFI, but achievement of WHO recommendations was more common (Table 7.2). Around half of participants expressed within 6 hrs of birth (WHO) but only 21% expressed within 2 hours of birth (UNICEF BFI). Fewer

<sup>38</sup>The start of one episode to the start of the prior episode

<sup>39</sup>Session yield divided by the number of hours from the start of the session to the start of the prior session

than half of participants achieved international recommendations for expressing frequency and longest gap between sessions, on any given day.

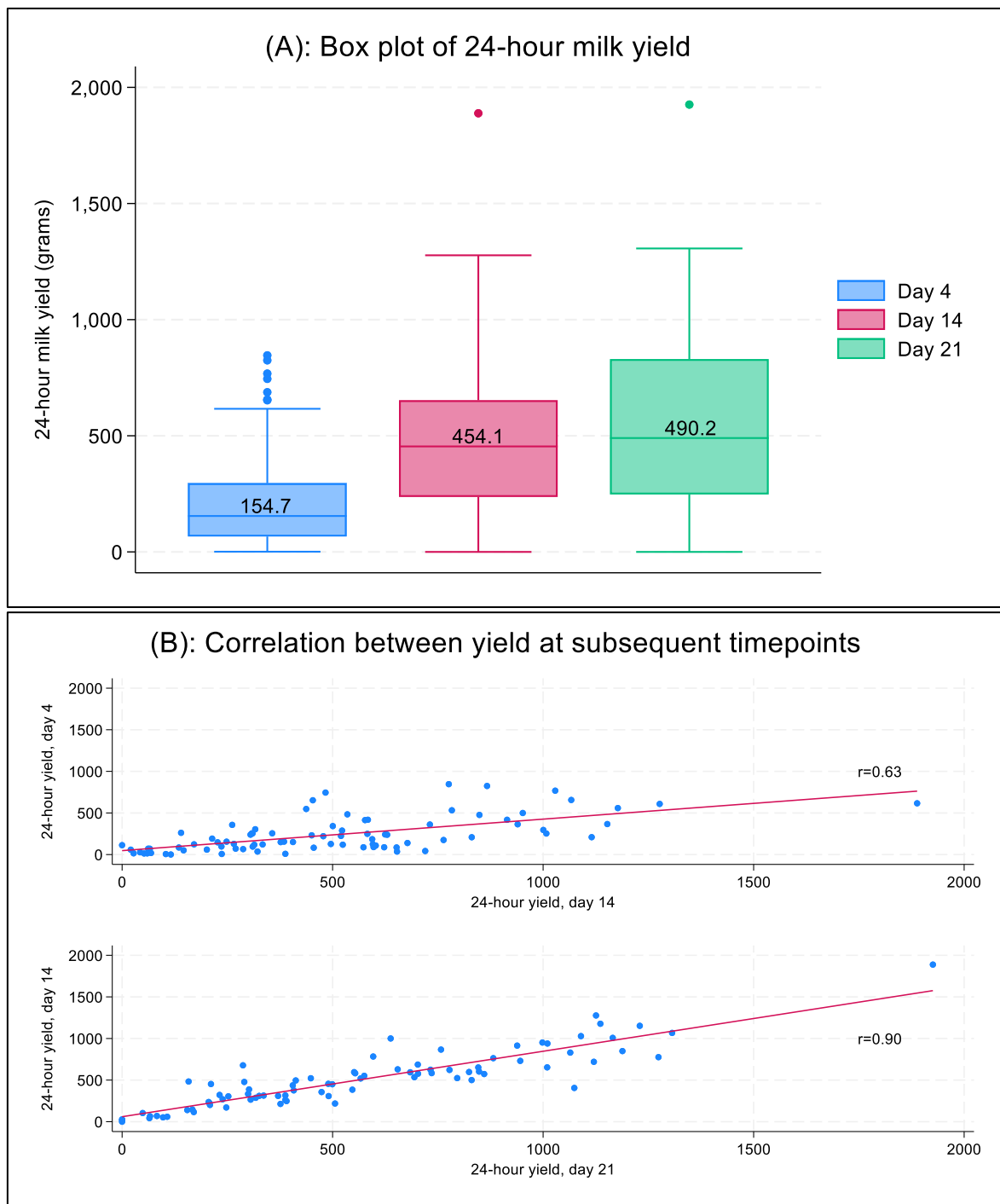
**Table 7.2: Adherence to international expressing recommendations**

|                                                | Timepoint | Achieving the recommendation, n (%) |
|------------------------------------------------|-----------|-------------------------------------|
| <b>UNICEF UK BFI recommendations</b>           |           |                                     |
| First expression $\leq 2$ hours from birth     |           | 25/120 (20.8)                       |
| Expressing frequency $\geq 8$ per day          | Day 4     | 14/103 (13.6)                       |
|                                                | Day 14    | 19/91 (20.9)                        |
|                                                | Day 21    | 15/91 (16.5)                        |
| Longest gap between expressions $\leq 6$ hours | Day 4     | 27/102 (26.5)                       |
|                                                | Day 14    | 41/89 (46.1)                        |
|                                                | Day 21    | 36/87 (41.4)                        |
| 24-hour milk yield $\geq 750$ g                | Day 14    | 18/90 (20.0)                        |
|                                                | Day 21    | 27/90 (30.0)                        |
| <b>WHO recommendations</b>                     |           |                                     |
| First expression $\leq 6$ hours from birth     |           | 62/120 (51.7)                       |
| Expressing frequency $\geq 7$ per day          | Day 4     | 29/103 (28.2)                       |
|                                                | Day 14    | 37/91 (40.7)                        |
|                                                | Day 21    | 40/91 (44.0)                        |

## 7.5.2 Expressed milk yield

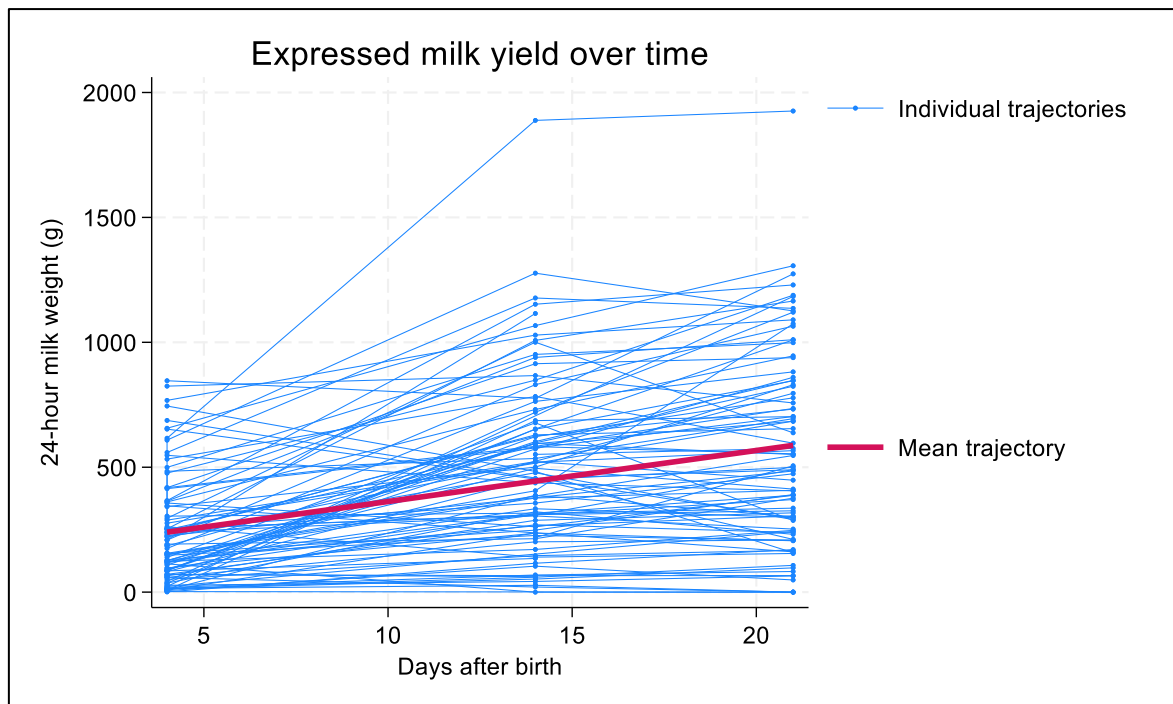
Milk yield increased over time and measurements were strongly correlated between days (Figure 7.2). This correlation was strongest between day 14 and 21 ( $r=0.90$ ). Day four yield was significantly lower in those participants who did not provide three complete logs compared to those who did (median 179.95g for 80 participants with complete logs, median 71g for 21 participants with incomplete logs,  $p=0.01$ ). Delayed secretory activation was noted in 31.7% of participants (32/101).

**Figure 7.2: (A) Expressed milk yield at each timepoint with median values identified. (B) Scatter plot of 24-hour yield on consecutive timepoints, with linear regression line and Pearson correlation coefficient**



The trajectories of milk yield were highly variable between individuals, as shown in Figure 7.3. Although there was an average linear increase over time, a third of the participants had lower milk yield at day 21 than at day 14 (24/83, 28.9%). A single outlier individual is notable in Figure 7.2 and Figure 7.3 at day 14 and 21.

**Figure 7.3: Individual and mean milk yield trajectories for all individuals**



### 7.5.3 Longer term breastmilk feeding

Table 7.3 shows the prevalence of breastmilk feeding at the three timepoints assessed. In the whole population, 64.9% (74/114) were exclusively breastmilk feeding at 36 weeks' PMA and 86.0% were giving any breastmilk (98/114). There was no evidence of a difference in baseline characteristics or feeding status at 36 weeks' PMA between participants recruited into the complete and abbreviated follow up phases.



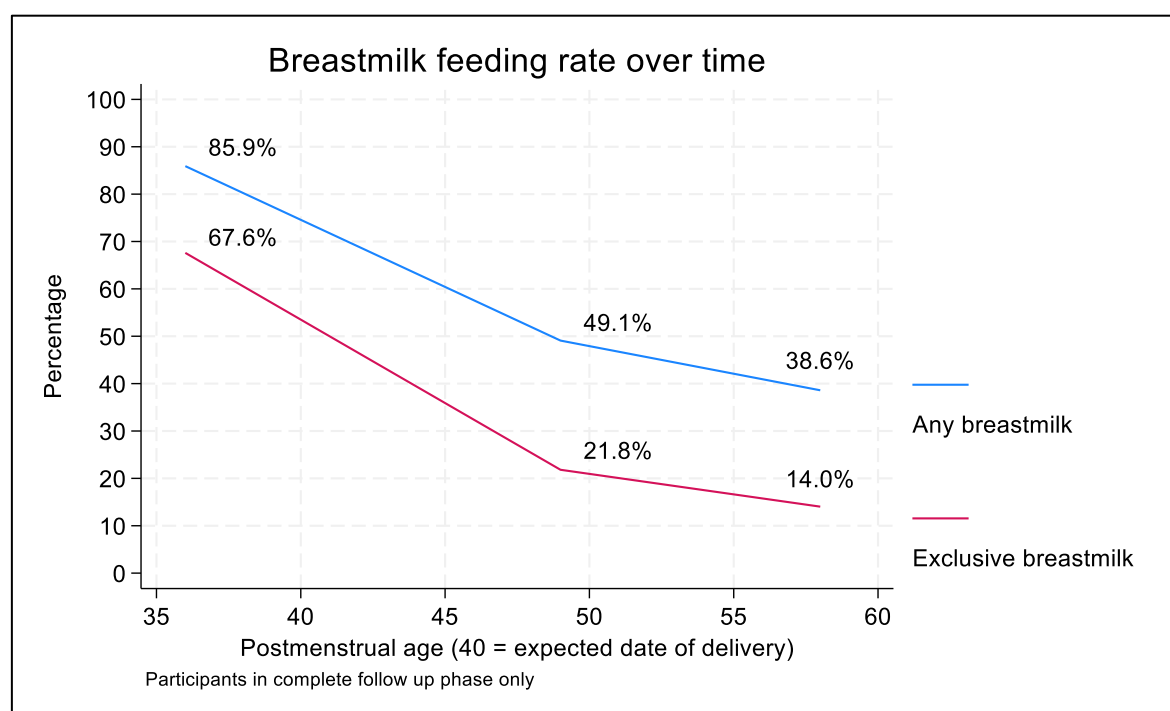
**Table 7.3: Breastmilk feeding outcomes over time**

|                                                       | Exclusive breastmilk<br>n/N, % (95% CI) | Any breastmilk<br>n/N, % (95% CI) | Infant<br>death <sup>†</sup> | Missing <sup>†</sup> |
|-------------------------------------------------------|-----------------------------------------|-----------------------------------|------------------------------|----------------------|
| <b>All participants</b><br>(n = 132 eligible)         |                                         |                                   |                              |                      |
| <b>36 weeks' PMA</b>                                  | 74/114<br>64.9% (55.4 – 73.6)           | 98/114<br>86.0% (78.2 – 91.8)     | 6                            | 12                   |
| <b>Long term follow up phase</b><br>(n = 85 eligible) |                                         |                                   |                              |                      |
| <b>36 weeks' PMA</b>                                  | 48/71<br>67.6% (55.5 – 78.2)            | 61/71<br>85.9% (75.6 – 93.0)      | 4                            | 10                   |
| <b>Two months' CA</b><br>(49 weeks' PMA)              | 12/55<br>21.8% (11.8 – 35.0)            | 27/55<br>49.1% (35.4 – 62.9)      | 4                            | 26                   |
| <b>Four months' CA</b><br>(58 weeks' PMA)             | 8/57<br>14.0% (6.3 – 25.8)              | 22/57<br>38.6% (26.0 – 53.4)      | 5                            | 23                   |

Binomial confidence intervals. <sup>†</sup>Not included in denominators. CI = confidence intervals. PMA = post-menstrual age. CA = corrected age

By four months' corrected age (CA), exclusive breastmilk had decreased to 14.0% (8/57) and any breastmilk to 38.6% (22/57; Table 7.3 and Figure 7.4). Infants were a mean of 8.2 weeks, 4.8 months and 6.9 months' actual age at the three timepoints, respectively.

**Figure 7.4: Breastmilk feeding prevalence in participants eligible for complete follow up only**



#### **7.5.4 Potential bias from missing data and data source**

As planned in the main RCT protocol (Chapter 5), infant feeding status could be included from two direct sources and one indirect source. The direct sources were participant response to an SMS or, at 36 weeks' only, extraction from the infant medical notes. The indirect source was knowledge of prior report of lactation cessation (and therefore included as current lack of any breastmilk feeding). At 36 weeks' PMA, the indirect route was the source of data for three participants. At two and four months' corrected age this was eight and nine participants respectively. Appendix Table J.13 and Appendix Figure J.3 show the trajectory of feeding outcomes over time using only direct sources of data in comparison to all sources.

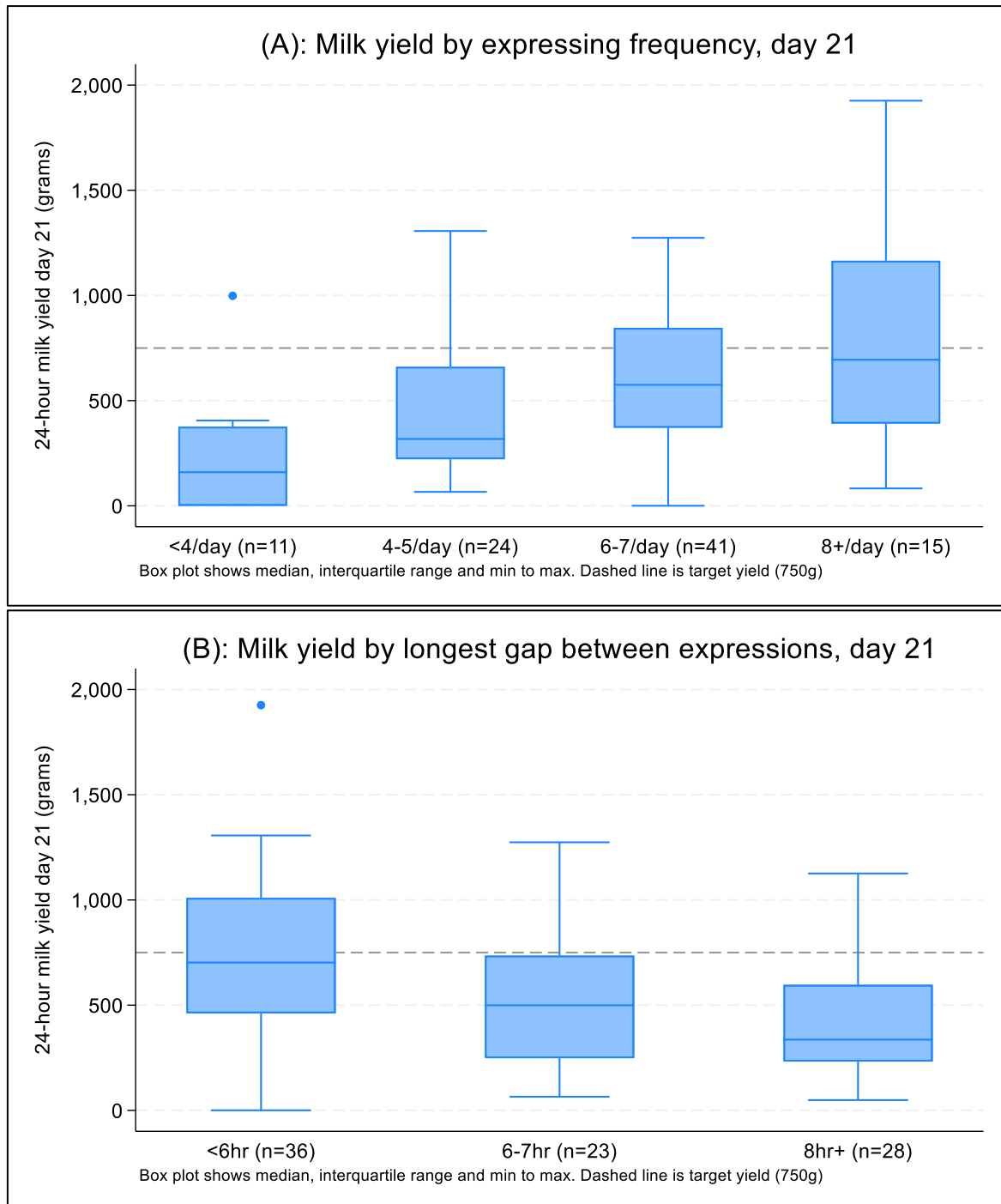
There were 53 participants with complete data for feeding status at all three timepoints. At 36 weeks' PMA, 81% of these participants were giving any breastmilk (43/53), compared to 100% of those with incomplete data (18/18,  $p=0.047$ ). For exclusive breastmilk there was no evidence of a difference (64%, 34/53 for the complete group compared to 78%, 14/18 in the incomplete group,  $p=0.3$ ). There was no evidence of differences in baseline characteristics between these two groups.

Considering only the 43 participants with a direct data source for their feeding status at all three timepoints, there were significant differences. At 36 weeks' PMA, these participants with complete direct data had a higher rate of any breastmilk (98%, 42/43) than those with incomplete direct data (73%, 19/26,  $p=0.002$ ). The same was true for exclusive breastmilk (79%, 34/43 in the complete group and 54%, 14/26 in the incomplete group,  $p=0.03$ ). Participants with direct data source at all three timepoints were also more likely to be living with a partner (40/43, 95%) compared to those with incomplete direct data (29/42, 78%;  $p=0.03$ ).

#### **7.5.5 Associations of baseline and expressing characteristics with milk yield**

The relationship of baseline characteristics and milk yield are described in Appendix Table J.14. Figure 7.5 shows day 21 milk yield according to expressing frequency and longest gap between expressions.

**Figure 7.5: Day 21 expressed milk yield by expressing pattern**



750g is the UK UNICEF Baby Friendly Initiative clinical recommendation for target expressed milk yield by day 14

Expressing frequency was positively correlated with milk yield ( $r=0.31$  at day 4,  $0.28$  at day 14,  $0.47$  at day 21; all  $p<0.05$ ). The longest gap between expressions was negatively correlated with milk yield ( $r= -0.35$

at day 4, -0.28 at day 14, -0.30 at day 21; all  $p < 0.05$ ). Expressing frequency and longest gap were strongly negatively correlated with each other ( $r = -0.78$  at day 21).

### 7.5.6 Determinants of exclusive breastmilk feeding

The following analyses address questions one and two identified in Section 7.3.4. In univariable analysis, there was no evidence of association between expressing frequency, longest gap between expressions or expressing duration with the outcome of exclusive breastmilk feeding at 36 weeks' PMA. However, 24-hour milk yield at day 4, day 14 and day 21 were all associated with exclusive breastmilk feeding at 36 weeks' PMA (Table 7.4).

**Table 7.4: Unadjusted relative risk for the outcome of exclusive breastmilk at 36 weeks' PMA**

|                                 | Relative Risk (unadjusted)<br>(95% CI) | p value      |
|---------------------------------|----------------------------------------|--------------|
| <b>Day 4</b>                    |                                        |              |
| Expressing frequency            | 1.02 (0.96 – 1.09)                     | 0.53         |
| Longest gap (per hour)          | 0.99 (0.96 – 1.02)                     | 0.48         |
| Expressing duration (per hour)  | 1.00 (0.90 – 1.12)                     | 0.99         |
| Expressed milk yield (per 100g) | <b>1.11 (1.04 – 1.19)</b>              | <b>0.001</b> |
| <b>Day 14</b>                   |                                        |              |
| Expressing frequency            | 1.02 (0.93 – 1.11)                     | 0.68         |
| Longest gap (per hour)          | 0.98 (0.93 – 1.03)                     | 0.34         |
| Expressing duration (per hour)  | 0.96 (0.84 – 1.10)                     | 0.58         |
| Expressed milk yield (per 100g) | <b>1.07 (1.01 – 1.13)</b>              | <b>0.02</b>  |
| <b>Day 21</b>                   |                                        |              |
| Expressing frequency            | 1.05 (0.97 – 1.14)                     | 0.26         |
| Longest gap (per hour)          | 1.00 (0.98 – 1.03)                     | 0.97         |
| Expressing duration (per hour)  | 1.00 (0.90 – 1.12)                     | 0.95         |
| Expressed milk yield (per 100g) | <b>1.06 (1.01 – 1.12)</b>              | <b>0.01</b>  |

### 7.5.7 Determinants of 24-hour milk yield at day four

#### 7.5.7.1 Univariable relationships

In univariable analysis, expressing frequency and longest gap between expressions were each significantly associated with the outcome of 24-hour milk yield at day four (Table 7.5). Both showed a linear trend so could be used as continuous variables. Each extra increment of expressing frequency was associated with 29.7g higher yield (95% CI 11.8 to 47.7) and each extra hour of longest gap between expressions was

associated with 12.7g lower yield (95% CI -19.5 to -5.9). There was no evidence for an association of total expressing duration with 24-hour yield.

By contrast, only one baseline variable was significantly associated with higher 24-hour milk yield on day four, which was prior breastmilk feeding experience of six months or more compared to those with less than six months experience (including those who were primiparous). Table 7.5 also reports notable baseline variables with no significant association with milk yield.

Potential confounders related to the method of expression were significantly associated with 24-hour yield (exclusive use of electric pump, exclusive simultaneous expression and expressing within six hours of birth were all associated with higher yield). The final potential expressing-related confounder of skin-to-skin contact duration was not significantly associated with 24-hour yield.

#### *7.5.7.2 Multivariable relationships*

Because of the high correlation between expressing frequency and longest gap between expressions, only one of these two variables was used in multivariable analysis (Table 7.5). Expressing frequency was chosen because of caution over the assumptions required to derive longest gap. The association of expressing frequency with 24-hour milk yield on day four persisted after adjustment for confounders (time to first expression, exclusive use of an electric pump, exclusive simultaneous expression and longer prior breastmilk feeding experience). There were minimal changes in the coefficients for any of the variables included in this multivariable model after adjustment.

This multivariable model included 99 participants and had a coefficient of determination (R-squared) of 43.5%, meaning that 56.5% of the variability in day four milk yield remained unexplained. In univariable analysis, expressing frequency accounted for 9% of the variability in 24-hour milk yield; prior breastmilk feeding experience accounted for 16%.

**Table 7.5: Unadjusted & adjusted coefficients for the outcome of 24-hour milk yield at day four**

|                                                                       | Unadjusted coefficients<br>(univariable) |                  | Adjusted coefficients<br>(multivariable) <sup>40</sup> |                  |
|-----------------------------------------------------------------------|------------------------------------------|------------------|--------------------------------------------------------|------------------|
|                                                                       | 24-hour milk yield in<br>grams (95% CI)  | p value          | 24-hour milk yield in<br>grams (95% CI)                | p value          |
| <b>Key expressing variables</b>                                       |                                          |                  |                                                        |                  |
| Expressing frequency                                                  | <b>29.7 (11.8 to 47.7)</b>               | <b>&lt;0.001</b> | <b>30.5 (15.7 to 45.3)</b>                             | <b>&lt;0.001</b> |
| Longest gap (per hr)                                                  | <b>-12.7 (-19.5 to -5.9)</b>             | <b>&lt;0.001</b> | -                                                      |                  |
| Expressing duration (per hr)                                          | 23.8 (-5.3 to 52.9)                      | 0.11             | -                                                      |                  |
| <b>Expressing-related potential confounders</b>                       |                                          |                  |                                                        |                  |
| First expression ≤6 hours<br>from birth                               | <b>88.3 (7.1 to 169.4)</b>               | <b>0.03</b>      | <b>90.1 (24.9 to 155.3)</b>                            | <b>0.007</b>     |
| Electric pump (versus<br>manual/hand/ combination)                    | <b>115.7 (24.0 to 207.4)</b>             | <b>0.01</b>      | <b>116.6 (40.9 to 192.3)</b>                           | <b>0.003</b>     |
| Simultaneous expression<br>(versus single/sequential/<br>combination) | <b>107.2 (7.6 to 206.8)</b>              | <b>0.04</b>      | <b>112.7 (29.6 to 195.7)</b>                           | <b>0.008</b>     |
| Skin to skin contact (per hr)                                         | 19.2 (-15.6 to 54.1)                     | 0.28             | -                                                      |                  |
| <b>Baseline potential confounders</b>                                 |                                          |                  |                                                        |                  |
| Prior breastfeeding ≥6mths<br>(versus <6mths or<br>primiparous)       | <b>202.5 (108.8 to 296.2)</b>            | <b>&lt;0.001</b> | <b>204.3 (125.2 to 283.3)</b>                          | <b>&lt;0.001</b> |
| Caesarean birth                                                       | -44.0 (-127.1 to 39.2)                   | 0.30             | -                                                      |                  |
| Birth gestation (per week)                                            | -5.7 (-22.9 to 11.6)                     | 0.52             | -                                                      |                  |
| Multiple birth                                                        | 71.8 (-40.5 to 184.1)                    | 0.21             | -                                                      |                  |
| Maternal age (per 10yr)                                               | -26.1 (-97.2 to 45.0)                    | 0.47             | -                                                      |                  |
| Left full time education ≥18<br>years                                 | 28.5 (-63.6 to 120.6)                    | 0.54             | -                                                      |                  |
| Intention to exclusively<br>breastmilk feed                           | 33.0 (-57.5 to 123.6)                    | 0.47             | -                                                      |                  |

### 7.5.8 Determinants of 24-hour milk yield at day 14 and 21

The relationship of the key expressing variables were also assessed with the outcome of 24-hour yield at day 14 and 21. Both expressing frequency and longest gap between expressions were significantly associated with 24-hour yield at day 14 and 21 in univariable analysis, with increasing coefficient size over time (Table 7.6).

<sup>40</sup>Adjusted for all variables included in this column

**Table 7.6: Unadjusted coefficients for key expressing variables with the outcome of 24-hour milk yield at day 4, 14 and 21**

|                             | Day 4                    |         | Day 14                   |         | Day 21                   |         |
|-----------------------------|--------------------------|---------|--------------------------|---------|--------------------------|---------|
|                             | 24-hour yield g (95% CI) | p value | 24-hour yield g (95% CI) | p value | 24-hour yield g (95% CI) | p value |
| <b>Expressing frequency</b> | 29.7<br>(11.8 to 47.7)   | 0.001   | 50.3<br>(14.4 to 86.2)   | 0.007   | 85.7<br>(51.2 to 120.3)  | <0.001  |
| <b>Longest gap (per hr)</b> | -12.7<br>(-19.5 to -5.9) | <0.001  | -23.3<br>(-40.6 to -5.9) | 0.009   | -29.7<br>(51.2 to 120.3) | <0.001  |

Table 7.7 shows unadjusted and adjusted coefficients for analysis of 24-hour milk yield at day 21. In comparison to day 4, key differences were that time to first expression, type of expression and simultaneous expression were not significantly associated with yield on day 21. The only confounder remaining in the multivariable model with expressing frequency was prior breastmilk feeding experience. The coefficients for expressing frequency and prior breastmilk feeding experience increased after adjustment.

This multivariable model included 90 participants and had a coefficient of determination (R-squared) of 35.6%, meaning that 64.4% of the variability in day 21 milk yield remained unexplained. In univariable analysis, expressing frequency accounted for 22% of the variability in 24-hour milk yield; prior breastmilk feeding experience accounted for 10%

**Table 7.7: Unadjusted & adjusted coefficients for the outcome of 24-hour milk yield at day 21**

|                                                                 | Unadjusted coefficients<br>(univariable) |                  | Adjusted coefficients<br>(multivariable) <sup>41</sup> |                  |
|-----------------------------------------------------------------|------------------------------------------|------------------|--------------------------------------------------------|------------------|
|                                                                 | 24-hour milk yield<br>grams (95% CI)     | p value          | 24-hour milk yield<br>grams (95% CI)                   | p value          |
| <b>Key expressing variables</b>                                 |                                          |                  |                                                        |                  |
| Expressing frequency                                            | <b>85.7 (51.2 to 120.3)</b>              | <b>&lt;0.001</b> | <b>94.4 (62.7 to 126.2)</b>                            | <b>&lt;0.001</b> |
| Longest gap (per hr)                                            | <b>-29.7 (-50.4 to -9.1)</b>             | <b>0.005</b>     | -                                                      |                  |
| Expressing duration (per hr)                                    | <b>88.7 (35.4 to 141.9)</b>              | <b>0.001</b>     | -                                                      |                  |
| <b>Expressing-related potential confounders</b>                 |                                          |                  |                                                        |                  |
| First expression ≤6 hours from birth                            | 73.6 (91.4 to 238.7)                     | 0.38             | -                                                      |                  |
| Electric pump (versus manual/hand/ combination)                 | 108.2 (-134.6 to 351.1)                  | 0.38             | -                                                      |                  |
| Simultaneous expression (versus single/sequential/ combination) | 144.8 (-73.6 to 363.4)                   | 0.19             | -                                                      |                  |
| Skin to skin contact (per hr)                                   | 41.4 (-4.5 to 87.4)                      | 0.08             | -                                                      |                  |
| <b>Baseline potential confounders</b>                           |                                          |                  |                                                        |                  |
| Prior breastfeeding ≥6mths (compared to <6mths or primiparous)  | <b>278.5 (98.5 to 458.4)</b>             | <b>0.003</b>     | <b>335.9 (181.8 to 490.1)</b>                          | <b>&lt;0.001</b> |
| Caesarean birth                                                 | 32.4 (-133.6 to 198.4)                   | 0.70             | -                                                      |                  |
| Birth gestation (per week)                                      | -7.6 (-42.0 to 26.7)                     | 0.66             | -                                                      |                  |
| Multiple birth                                                  | 192.5 (-28.3 to 413.4)                   | 0.09             | -                                                      |                  |
| Maternal age (per 10yr)                                         | 47.9 (-112.6 to 208.4)                   | 0.56             | -                                                      |                  |
| Left full time education ≥18 years                              | <b>301.5 (132.5 to 470.5)</b>            | <b>0.001</b>     | -                                                      |                  |
| Intention to exclusively breastmilk feed                        | 144.0 (-35.2 to 323.1)                   | 0.11             | -                                                      |                  |

### 7.5.9 Assessing clinical recommendations on expressing pattern

The following analyses address question three identified in Section 7.3.4. Table 7.8 shows expressing frequency and longest gap at day 4 and day 21 with reference to 24-hour milk yield on each of these days. Cut-offs for expressing frequency and longest gap were constructed with reference to UNICEF BFI recommendations. On day 4, adjustment was for the variables reported in the multivariable model shown in Table 7.5. On day 21, adjustment was for prior breastmilk feeding experience.

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<sup>41</sup>Adjusted for all variables included in this column



**Table 7.8: Adjusted coefficients for UNICEF BFI clinical recommendations with the outcome of 24-hour yield on day 4 and 21**

| Day 4                       |                                                    |              |    | Day 21                                             |                  |    |
|-----------------------------|----------------------------------------------------|--------------|----|----------------------------------------------------|------------------|----|
|                             | 24-hour milk yield in grams (95% CI) <sup>42</sup> | p value      | n  | 24-hour milk yield in grams (95% CI) <sup>43</sup> | p value          | n  |
| <b>Expressing frequency</b> |                                                    |              |    |                                                    |                  |    |
| ≥8/day                      | [baseline]                                         |              | 14 | [baseline]                                         |                  | 15 |
| <8/day                      | <b>-124.6 (-221.5 to -27.8)</b>                    | <b>0.01</b>  | 89 | <b>-268.1 (-470.3 to -65.8)</b>                    | <b>0.01</b>      | 76 |
| <b>Expressing frequency</b> |                                                    |              |    |                                                    |                  |    |
| ≥8/day                      | [baseline]                                         |              | 14 | [baseline]                                         |                  | 15 |
| 6-7/day                     | -82.1 (-190.1 to 25.9)                             | 0.14         | 30 | -163.8 (-369.6 to 42.0)                            | 0.12             | 41 |
| <6/day                      | <b>-146.8 (-246.1 to -47.4)</b>                    | <b>0.004</b> | 59 | <b>-402.1 (-615.4 to -188.8)</b>                   | <b>&lt;0.001</b> | 35 |
| <b>Longest gap</b>          |                                                    |              |    |                                                    |                  |    |
| ≤6 hours                    | [baseline]                                         |              | 27 | [baseline]                                         |                  | 37 |
| >6 hours                    | <b>-91.4 (-168.4 to -14.3)</b>                     | <b>0.03</b>  | 75 | <b>-249.1 (-393.4 to -104.7)</b>                   | <b>0.001</b>     | 51 |
| <b>Longest gap</b>          |                                                    |              |    |                                                    |                  |    |
| <6 hours                    | [baseline]                                         |              | 27 | [baseline]                                         |                  | 36 |
| 6-7 hours                   | -41.1 (-137.5 to 55.3)                             | 0.40         | 23 | <b>-221.0 (-396.8 to -45.2)</b>                    | <b>0.01</b>      | 23 |
| ≥8 hours                    | <b>-114.1 (-195.0 to -33.3)</b>                    | <b>0.006</b> | 52 | <b>-294.6 (-462.3 to -126.9)</b>                   | <b>0.001</b>     | 28 |

As binary variables, the clinical recommendation for longest gap between expressions (less than six hours) and expressing frequency (at least eight per day) were associated with significantly higher 24-hour milk yield at both timepoints.

As a three-category variable, expressing eight or more times per day was associated with significantly higher yield than expressing less than six times per day, but not in comparison to expressing 6-7 times per day, at both timepoints.

At day 21, a longest gap of less than six hours was associated with higher 24-hour yield than both a longest gap of 6-7 hours and a longest gap of eight hours or more. In contrast, at day 4, a longest gap of less than six hours was associated with higher 24-hour yield than a longest gap of eight hours or more, but not the intermediate category of 6-7 hours.

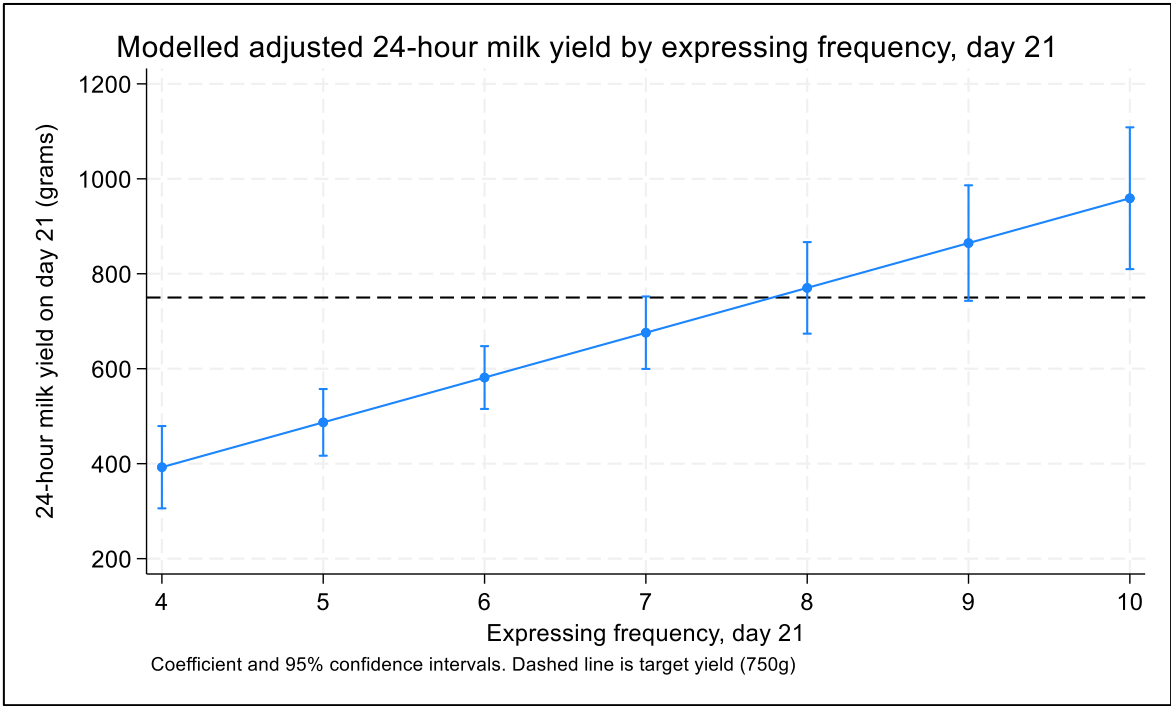
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<sup>42</sup>Adjusted for first expression after birth, pump mode, simultaneous expression and prior breastmilk feeding experience

<sup>43</sup>Adjusted for prior breastmilk feeding experience

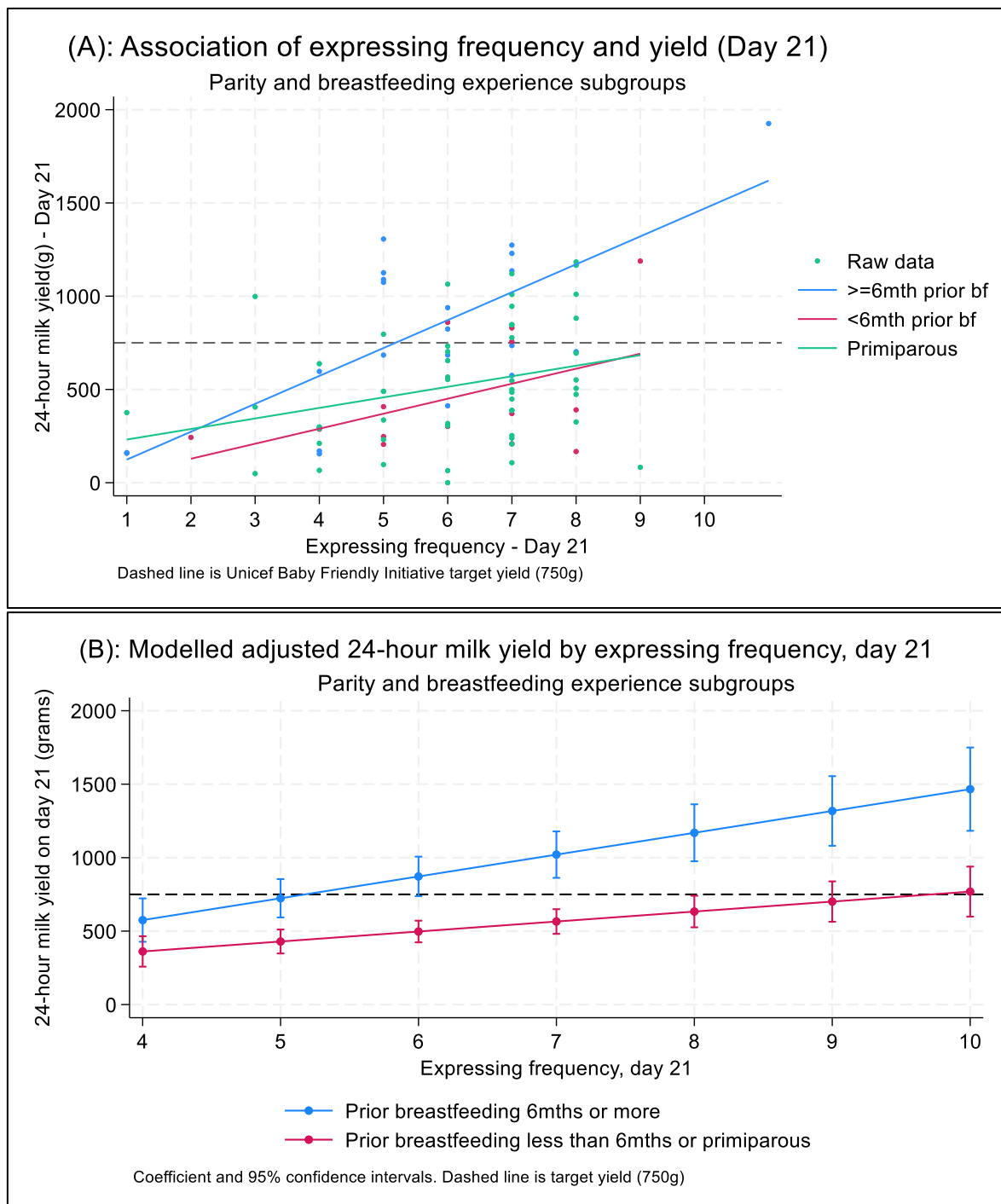
Clinical recommendations can also be assessed by using the multivariable statistical model to predict the adjusted 24-hour milk yield across a range of expressing frequencies. This is shown for day 21 with reference to the UNICEF BFI 24-hour yield target of 750g (Figure 7.6). The model is adjusted for prior breastfeeding experience. This demonstrates that participants expressing eight times per day achieved an adjusted 24-hour yield at around the target yield, but there is considerable overlap in confidence intervals in the range of 7-9 times per day.

**Figure 7.6: Adjusted 24-hour milk yield by expressing frequency at day 21**



Given the very large effect estimate for the non-modifiable factors of prior breastfeeding experience on milk yield in both multivariable models presented above, a post-hoc interaction test was performed with expressing frequency and prior breastmilk feeding experience of six months or more ( $p=0.02$ ). The association of 24-hour yield and expressing pattern is presented for these subgroups in Figure 7.7. Although the interaction variable combines primiparous individuals with multiparous individuals with less than six months of prior breastmilk feeding experience, these two groups are shown separately in the raw data in this graph to demonstrate their similarity. People with more than 6 months of prior breastfeeding experience achieved target yield with a much lower average expressing frequency than others.

**Figure 7.7: Actual (A) and modelled (B) 24-hour milk yield by expressing frequency at day 21 according to prior breastmilk feeding experience**



#### 7.5.10 Session-level variables as determinants of hourly milk yield

The following analyses address questions two and four identified in Section 7.3.4. The relationship of expressing session-level variables with the outcome of hourly milk yield at day 4, 14 and 21 was assessed

using multilevel modelling on each individual day (an approach described in Figure 7.1). In univariable analysis, the gap to prior session was significantly negatively associated with hourly milk yield at all timepoints (Table 7.9).

There were no other consistent associations of session-level variables with hourly milk yield in univariable analysis. On day 14 only, session timing of 7am to 3pm was associated with higher hourly milk yield than 11pm to 7am (3.2g/hr, 95% CI 0.7 to 5.8), and simultaneous expression was associated with higher hourly milk yield (14.5g/hr, 95% CI 5.0 to 24.1). On day 4 only, electric pumping was associated with higher hourly milk yield than other expressing modes (7.9g/hr, 95% CI 4.9 to 10.9).

**Table 7.9: Unadjusted association with the outcome of hourly milk yield at day 4, 14 & 21**

|                                             | Day 4                         |                  | Day 14                        |                  | Day 21                        |                  |
|---------------------------------------------|-------------------------------|------------------|-------------------------------|------------------|-------------------------------|------------------|
|                                             | Hourly yield in g/hr (95% CI) | p value          | Hourly yield in g/hr (95% CI) | p value          | Hourly yield in g/hr (95% CI) | p value          |
| <b>Gap to prior session (hours)</b>         | <b>-1.0 (-1.3 to -0.8)</b>    | <b>&lt;0.001</b> | <b>-2.2 (-2.6 to -1.8)</b>    | <b>&lt;0.001</b> | <b>-2.5 (-3.1 to -2.0)</b>    | <b>&lt;0.001</b> |
| <b>Session duration (per 10 mins)</b>       | 0.5 (-0.4 to 1.5)             | 0.25             | 0.0 (-1.6 to 1.6)             | 0.98             | 0.2 (-1.7 to 2.1)             | 0.89             |
| <b>Session timing</b>                       |                               |                  |                               |                  |                               |                  |
| ≥0700 to 1500                               | 0.5 (-1.7 to 2.7)             | 0.65             | <b>3.2 (0.7 to 5.8)</b>       | <b>0.01</b>      | 2.6 (-0.7 to 5.8)             | 0.12             |
| ≥1500 to 2300                               | 1.1 (-1.0 to 3.2)             | 0.32             | 1.4 (-1.1 to 3.9)             | 0.29             | 3.2 (-0.06 to 6.4)            | 0.05             |
| ≥2300 to 0700                               | [baseline]                    |                  | [baseline]                    |                  | [baseline]                    |                  |
| <b>Electric pump<sup>44</sup></b>           | <b>7.9 (4.9 to 10.9)</b>      | <b>&lt;0.001</b> | 4.6 (-3.6 to 12.8)            | 0.27             | 0.3 (-7.3 to 7.9)             | 0.94             |
| <b>Simultaneous expression<sup>45</sup></b> | 4.6 (-0.1 to 9.3)             | 0.06             | <b>14.5 (5.0 to 24.1)</b>     | <b>0.003</b>     | 7.0 (-2.7 to 16.7)            | 0.16             |

Adjusted analysis is shown in Appendix Table J.15. Session timing and simultaneous expression were no longer associated with hourly milk yield on day 14 when prior breastfeeding experience was adjusted for. In contrast, expressing session duration, use of an electric pump and simultaneous expression were positively associated with hourly milk yield at day 4 in adjusted analysis.

<sup>44</sup> compared to hand, manual or mixture

<sup>45</sup> compared to single or sequential

## 7.5.11 Sensitivity analyses

### 7.5.11.1 *Removing high yield outlier*

There was a single participant who was an outlier in several characteristics – for example milk yield (exceeding 1.8 litres by day 14), expressing duration (more than 7 hours per day at each timepoint) and expressing frequency (10 or more times per day at each timepoint). This participant had more than six months of prior breastfeeding experience and multiple birth. When this participant was removed from the analysis of 24-hour yield, the adjusted coefficient for breastfeeding experience reduced in size but only by a small amount. On day 4 this was a reduction from 204.3g (95% CI 125.2 to 283.3) to 193.7g (95% CI 113.9 to 273.5). On day 21 this was a reduction from 335.9g (181.8 to 490.1) to 297.3g (142.1 to 452.4).

There were some changes in the categorical analyses presented in Table 7.8; all coefficients reduced in size, with a greater change seen for expressing frequency than longest gap between expressions (Appendix Table J.16). Expressing eight or more times per day was no longer significantly associated with higher 24-hour milk yield at day 21 than expressing less than eight times. The other analyses became less significant (but remaining within the same divisions of significance related to the threshold of  $p < 0.05$ ).

### 7.5.11.2 *Excluding incomplete data*

It was noted that individuals who submitted complete logs (day 4, 14 and 21) had a higher day four milk yield than those with incomplete logs (predominantly submitting day four only). There were 19 participants included in day four multivariable analysis with incomplete logs for other timepoints. When these participants were removed there were no meaningful differences in the results of analyses (Appendix Table J.17). There was slight reduction of precision as expected due to reduction in the sample size.

### 7.5.11.3 *Limiting the assumptions of deriving the gap prior to the first logged session*

For the first logged session of each measurement day, the gap to prior session was derived as described in section 7.4.1. The affected first sessions represented the longest gap in the 24-hour period for 47.1%

of day 4 logs (48/102) and 37.9% (33/87) of day 21 logs. First logged sessions had significantly greater milk yield on day 21 (median difference 34.6g,  $p=0.001$ ) and significantly greater gap to prior session on both days (day 21 median difference 3.0 hours,  $p<0.001$ ) compared to other sessions. Session duration was not different between first logged and other sessions.

This predominance of the first session as the longest gap in the 24 hour period means that excluding the first logged sessions as a sensitivity analysis has a large impact on this variable. On day 4, the number of participants with a longest gap of more than 6 hours would rise from 27/102 (26.5%) with the derivation assumption to 50/92 (54.4%) without the assumption. On day 21 it would rise from 37/87 (42.5%) to 58/84 (69.1%).

Without the derivation assumption there were significant impacts on the univariable regression analysis related to longest gap between expressions. On day 4, the coefficient for longest gap changed from -12.7g (95% CI -19.5 to -5.9,  $n = 101$ ) with the assumption to -11.0g (95% CI -28.1 to 6.2,  $n = 92$ ) without the assumption. On day 21, the coefficient changed from -29.7g (95% CI -50.4 to -9.1,  $n = 86$ ) to -15.3g (-52.9 to 22.4,  $n = 84$ ). Longest gap was not included in multivariable modelling so there was no impact on adjusted analyses. There were major changes in the categorical analyses presented in Table 7.8 related to longest gap; all coefficients reduced in size and only one out of the six statistical inferences remained significant at  $p<0.05$  (Appendix Table J.18).

In contrast, at the expressing session level with an outcome of hourly milk yield, the coefficient for the gap to prior session increased in this sensitivity analysis. On day 4, the coefficient for gap to prior session increased from -1.0g/hr (95% CI -1.3 to -0.8,  $n = 471$  sessions,  $n = 101$  people) with the assumption to -1.5g/hr (95% CI -2.0 to -1.0,  $n = 401$  sessions,  $n = 92$  people) without the assumption. On day 21, the coefficient increased from -2.5g/hr (95% CI -3.1 to -2.0,  $n = 479$  sessions,  $n = 86$  people) to -3.4g/hr (95% CI -4.2 to 2.6,  $n = 433$  sessions,  $n = 84$  people).

## 7.6 Summary

Table 7.10 summarises these results with reference to the original research questions.

A social media infographic with the aim of disseminating the results to parents and the public is provided in Appendix K, which was created with trial participants as outlined in Chapter 4.

**Table 7.10: Brief answers to the pre-specified exploratory research questions**

| Specific questions                                                                                                                                                                                                                            | Summary of evidence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>1. Is expressing frequency associated with contemporaneous 24-hour milk yield and/or long-term breastmilk feeding outcomes? Does any association change with increasing time from birth?</b>                                               | <p>Expressing frequency was strongly associated with 24-hour expressed milk yield but not with long-term exclusive breastmilk feeding.</p> <p>The size of the positive association of expressing frequency with milk yield increased from day 4 to 21.</p>                                                                                                                                                                                                                                                                                                                                   |
| <b>2. Is the gap between expressions, and/or the longest gap in the 24-hour period, associated with contemporaneous milk yield and/or long-term breastmilk feeding outcomes? Does any association change with increasing time from birth?</b> | <p>The gap to prior expression was negatively associated with hourly milk yield at a session level.</p> <p>The longest gap between expressions was associated with 24-hour milk yield but not long-term exclusive breastmilk feeding. This finding was not robust to sensitivity analysis related to assumptions required to assign a gap to the first logged session.</p> <p>The size of the negative association of longest gap between expressions with milk yield, and of the gap to prior expression with hourly milk yield at a session level, increased from day 4 to day 21.</p>     |
| <b>3. Are current clinical recommendations for expressing frequency and longest gap associated with good lactation outcomes?</b>                                                                                                              | <p>Expressing eight or more times a day was associated with higher 24-hour milk yield than less than six times a day at day 4 and day 21. The data on expressing six to seven times a day in relation to these two categories was inconclusive.</p> <p>A longest gap between expressions of less than six hours was associated with higher 24-hour milk yield than longest gap greater than six hours, at day 4 and day 21.</p> <p>A post-hoc finding suggested that participants with more than six months of prior breastfeeding experience could express less frequently than others.</p> |
| <b>4. Is there a positive independent association of night-time circadian timing of expressing sessions once the gap between expressing sessions is adjusted for?</b>                                                                         | No                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |

## 7.7 Discussion

This study strongly supports the importance<sup>40,254,273,277</sup> of expressing frequency for the outcome of 24-hour expressed milk yield, with increasing impact over time. For example, at day 21 there was an adjusted increase in 24-hour yield of 94g per expressing session. In contrast, expressing frequency was not associated with exclusive breastmilk at 36 weeks' PMA, whereas expressed milk yield was strongly associated with this outcome. This may be because milk yield is the key factor for breastfeeding outcome and expressing frequency only explained 9% of the variability in milk yield on day 4 and 22% on day 21. This is important because it demonstrates that manipulating the expressing frequency alone may have some impact on short-term lactation outcomes but is likely to have more limited potential impact on long-term breastmilk feeding outcomes in this setting.

UK clinical recommendations to express at least eight times per day were supported because this was associated with achievement of recommended milk yield on average. However, there was wide inter-individual variability and few people expressed at this frequency. This report cannot conclusively say that expressing eight or more times a day was associated with higher 24-hour milk yield than expressing 6-7 times per day, which was closer to the median frequency and therefore more achievable for this cohort. However, participants expressing less than six times per day were unlikely to achieve target yields. This would be useful information for parents who feel that expressing eight times per day is unrealistic or unachievable.

We attempted to analyse the association of longest gap between expressions with 24-hour milk yield. A significant negative association was reported, with milk yield reducing by 30g for each hour increase of the longest gap between expressions on day 21. The clinical recommendation of having a gap no longer than six hours was supported. However, these findings were not robust to sensitivity analyses removing an outlier participant with very short longest gaps, or removing certain assumptions required to derive the gap to prior session for the first recorded session of each day. Therefore this finding should be interpreted with caution.



The session level (hourly milk yield) analysis reported here was able for the first time to separate two factors associated with the clinical recommendation to express in the middle of the night; the circadian timing and the gap to the prior expressing session. The analysis does not support an inherent value to the night-time period, once the gap to prior session is accounted for. Although parents would have to express in the night period to keep the longest expressing gap under the recommended six hours, this finding supports flexibility between individuals in exactly when to express, which could take into account their sleep pattern. This is important as sleep interruption is cited as a reason to stop expressing<sup>30</sup>.

Electric pumping and simultaneous expression were positively associated with 24-hour and hourly milk yield on day four, which is in line with UK clinical recommendations. Expressing duration was not consistently associated with milk yield. Expressing within the first six hours of very preterm birth was associated with milk yield, at least up to the second week after birth.

We have demonstrated a novel exploratory finding that participants with longer prior breastmilk feeding experience (in this study defined as more than six months) expressed on average less frequently to achieve the same yield as those who were primiparous or had shorter duration of prior breastmilk feeding. This could allow clinicians to individualise expressing recommendations. However, this was not a pre-specified hypothesis and is based on 26 participants with more than six months of breastmilk feeding experience. The slight under-representation of multiparous people in the recruited population in comparison to the eligible population (reported in Chapter 6), is a cause for concern over bias in the characteristics of the multiparous people who decided to take part. Therefore, the interaction of breastfeeding experience with expressing frequency should be interpreted cautiously and requires replication.

### **7.7.1 Setting the findings in context**

This cohort showed similar expressing frequency to other cohorts in the literature<sup>33,34,40,275,283</sup>. However, the frequency of delayed secretory activation was lower than other reports<sup>36,160</sup> and this study had a

shorter time to first expression (median six hours in this study compared to eight<sup>36</sup>, nine<sup>33,46</sup> and 20-24 hours<sup>277,284</sup> in other studies). Expressed milk yield was broadly similar to previous cohorts<sup>273,277,283,285</sup> although notably lower than two small studies that looked in depth at expressing dynamics<sup>36,40</sup>. This may suggest that our study recruited a broader range of participants rather than simply the most motivated.

London and Thames Valley neonatal units are known to have higher breastmilk rates at discharge for very preterm infants than the national average, and three out of four neonatal units are level three UNICEF BFI accredited, suggesting a good level of lactation support. The rates of any breastmilk at discharge in 2022 at the four recruiting units from prospective national audit data were 73% (Oxford), 78% and 85% (London) and 63% (Manchester). Weighted according to the number of recruits from each hospital, this would give an expected trial prevalence of 74% any breastmilk at discharge. The trial reported a rate of 87% (95% confidence interval of 79% to 92%) at 36 weeks' PMA (a slightly earlier timepoint than discharge for most infants of this gestation<sup>286</sup>).

In contrast, the national UK average for any breastmilk at discharge after very preterm birth was 63% in 2022<sup>235,287</sup> and the prevalence for very preterm infants at 36 weeks' PMA in unpublished data from the 2018 National Maternity Survey<sup>288</sup> was also 63%. Although the higher than average breastmilk feeding rates in this sample may reduce generalisability, it is useful for analysis of the relationship of expressing behaviour with breastfeeding outcomes.

The positive association of expressing frequency and milk yield we have found is consistent with most other studies<sup>38–41,43,76,254,273,275,276</sup>. One previous study showed a smaller adjusted increase in 24-hour yield of 25ml per expressing session (at average day 14)<sup>41</sup>. The correlation coefficients of expressing frequency and yield are very similar to those previously reported (0.32 to 0.51 in the literature<sup>38,39,43,275</sup>, compared to 0.28 to 0.47 in this study). The mean differences in milk yield for “high frequency expressing” (≥6-7 per day at 2-4 weeks after birth) in other studies<sup>39,40,273</sup> were very similar to the value of 268.1g when expressing ≥8 per day at 21 days found here. The lack of association of expressing frequency with longer term breastmilk feeding is part of a mixed picture in the literature, with a small number of studies

reporting no association<sup>38,276,277</sup> and a small number reporting association<sup>31,134,153</sup>. This may reflect context dependence, as decisions on long term breastmilk feeding are subject to multiple factors, including sociodemographic influences.

Only one study is known to have reported on the longest gap between expressions previously, finding no association with milk yield at two or four weeks after birth<sup>44</sup>. Mean longest gap was shorter in that report (6.5 hours) than seen here (7.5 to 10 hours depending on the timepoint). This supports the caution in interpretation of our findings on this variable. Our findings on electric pumping and simultaneous expression are consistent with the small number of randomised studies performed<sup>41,289</sup>.

In the NICU context, two previous studies with mothers of very preterm infants have shown a similar magnitude of increase in milk yield with longer previous breastfeeding experience; higher milk yield (by 222ml/day)<sup>42</sup> and 22-25% greater milk yield at week one and four<sup>134</sup>. One study with mixed gestation NICU mothers showed a median difference in duration of exclusive breastfeeding of one month for those with any previous breastfeeding experience<sup>290</sup>. In contrast, one study showed no correlation of previous breastfeeding experience with the ability to express at least 500g per day by week 5<sup>91</sup>.

It is possible that at least part of this finding is due to confounding, whereby people with longer length of prior breastmilk feeding are more motivated, have higher self-efficacy<sup>291</sup> and may put in place unmeasured supportive behaviours to achieve higher milk yield. However, there could also be an element of physiological difference in the breast tissue of someone who has experienced prolonged previous lactation that increases its efficiency.

In support of the latter idea, one study<sup>292</sup> has shown that milk production per unit of breast tissue is much higher after six months of lactation (for example 4g/ml at nine months compared to 2g/ml in the first six months). It is well established in mice that mammary epithelial cells show epigenetic changes after the first pregnancy/lactation cycle that causes earlier activation of milk-related genes for subsequent pregnancy/lactation cycles<sup>293,294</sup>. In multiparous rats, there is increased responsiveness to prolactin and therefore a lower circulating prolactin concentration<sup>295</sup>.

A similar phenomenon has been seen in two human studies. One showed lower circulating prolactin in the first four days after birth in multiparous women, but higher infant milk intake<sup>296</sup>. The second showed increased milk intake at day seven after the woman's second birth in comparison to their first birth, despite shorter total duration of feeding time<sup>297</sup>. In addition, increased length of prior breastfeeding has been linked with greater epithelial area and increased expression of progesterone receptors in clinical breast tissue samples after cessation of lactation<sup>298</sup>.

### **7.7.2 Strengths and Limitations**

The strengths of this study are the broad population of included participants, in terms of sociodemographic variables as well as expressing behaviour. The inclusion of individual and session level variables across a range of measurement days has yielded novel findings and will help clinicians to personalise the advice they offer to families.

The most significant limitation of the study is the sample size. Gathering a larger sample while retaining the level of detail on individual expressing sessions on multiple days was not possible within the doctoral timeline.

A further limitation of the study is the reliance on participant-measured data on expressing pattern and yield, which could affect data accuracy; and the restriction of data collection to three timepoints rather than a continuous log. Single day logs meant that some assumptions were required to derive the gap to prior session for the first expressing session of the day, which had a significant impact on the analysis conclusions related to the longest gap between expressions. The characteristics of the first expression of the day were significantly different from others so it is unclear whether the impact on the analysis was because of bias in the derivation assumption or because these expressing sessions have an important and differential influence on lactation outcomes.

Both elements of trial design (participant measurement and single day collection timepoints) were chosen after extensive parent involvement to reduce interference and burden for participants at a stressful time<sup>2</sup>

(as described in Chapter 4) and may have resulted in the broader representation of expressing behaviour and yield than seen in other detailed studies<sup>36,40</sup>.

There were several important variables that could not be included in this analysis that are known to be important for expressed milk outcomes; most notably perinatal factors such as chorioamnionitis<sup>37,154</sup>, assisted reproduction<sup>148,154,299</sup> and obesity<sup>60,154,300</sup>. The role of placental dysfunction in both the occurrence of preterm birth and on alveolar differentiation in the mammary glands<sup>60</sup> may mean that measures of placental function have particular relevance in this context. This could lead to residual confounding. The large amount of residual unexplained individual variation in 24-hour milk yield implies that influential variables may have been missing. However, it is unlikely that these perinatal variables would have dramatically reduced the residual variation; there are likely important influences that have not yet been identified, such as underlying genetic variation.

There was some loss to follow up as time went on, with a higher number of expressing logs submitted at day four than later timepoints. Potential bias was noted in the participants who stopped completing expressing logs after day four as they had lower day four yield than participants with complete logs. The conclusions from later analysis may therefore be less generalisable than the day four analysis, although removing participants with incomplete logs did not change the analysis conclusions at day four.

### **7.7.3 Conclusions**

Overall, the data in this study provides much needed evidence related to current clinical recommendations on expressing patterns. UK and international recommendations on expressing frequency and longest gap between expressions were supported, but more nuance was provided on mothers who may be able to relax these recommendations while maintaining good milk yield and a change in emphasis can now be suggested related to night-time expression.

## **7.8 Summary**

This chapter outlined the existing evidence available on the relationship of expressing pattern (frequency, gaps and timing) and lactation outcomes. The methods for exploring these relationships within our own cohort were presented, followed by the results and initial discussion of their implications. The following chapter is the final discussion, which summarises and discusses the thesis as a whole.

# Chapter 8: Discussion

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## 8.1 Chapter Overview

The overall aim of the thesis was to investigate determinants of the quantity of breastmilk that can be expressed by mothers of very preterm infants, and related breastfeeding outcomes, with particular focus on relaxation interventions and the pattern of expressing. To address this aim I have systematically reviewed the literature on relaxation and lactation, involved parents of preterm infants in planning the programme of research, undertaken a randomised controlled trial of relaxation and lactation after very preterm birth and performed an exploratory analysis on the relationship of expressing patterns with lactation outcomes.

This chapter provides an overview of the findings from this programme of work and discusses these findings in relation to the existing literature and the clinical context. It explores the strengths and limitations of the work presented, clinical implications and recommendations for future work integrating lessons learned.

## 8.2 Research rationale

In Chapter 2, I described the importance of maternal breastmilk to very preterm infants to reduce significant morbidity and mortality, and the difficulties that mothers encounter in expressing milk after very preterm birth. I noted that breastfeeding and expressing have been identified by the WHO, by priority setting partnerships and by researcher-led workshops as key priorities, but there are few randomised trials in the field and existing observational studies are difficult to apply to clinical practice. Qualitative evidence on the burden of current expressing recommendations and the connections between lactation and mental health after very preterm birth were explored. This explains why the programme of research is important and what gaps in the literature were to be addressed.

### 8.3 Summary of research findings

The key findings of the thesis are presented in relation to the thesis objectives (Table 8.1).

**Table 8.1: Key Findings of thesis, in relation to objectives**

| Objective                                                                                                                                                                                       | Key Findings (highest interest/importance)                                     | Effect Estimate (95% CI)                 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|------------------------------------------|
| To review current evidence on the effect of relaxation therapy on lactation and mental health outcomes in lactating mothers and their infants                                                   | <b>High certainty evidence:</b>                                                |                                          |
|                                                                                                                                                                                                 | Reduction in maternal stress                                                   | SMD -0.49<br>(-0.70 to -0.27)            |
|                                                                                                                                                                                                 | <b>Moderate certainty evidence:</b>                                            |                                          |
|                                                                                                                                                                                                 | Increase in milk quantity                                                      | SMD 0.73<br>(0.57 to 0.89)               |
|                                                                                                                                                                                                 | Reduction in maternal anxiety                                                  | SMD -0.47<br>(-0.66 to -0.29)            |
| To investigate the effect of relaxation therapy on breastmilk yield, breastfeeding duration and mental health outcomes in mothers of very preterm infants, using a randomised controlled design | <b>Primary Outcome:</b>                                                        |                                          |
|                                                                                                                                                                                                 | No change in 24-hour milk quantity                                             | Adjusted MD 73.9g<br>(-61.7 to 209.5)    |
|                                                                                                                                                                                                 | Higher 24-hour milk quantity with higher adherence (non-randomised comparison) | Adjusted MD 245.9g<br>(36.0 to 455.7)    |
|                                                                                                                                                                                                 | <b>Secondary Outcomes:</b>                                                     |                                          |
|                                                                                                                                                                                                 | No change in maternal anxiety                                                  | Adjusted MD -1.9<br>(-8.2 to 4.3)        |
|                                                                                                                                                                                                 | No change in maternal post-traumatic stress                                    | Adjusted Med Diff -1.4<br>(-11.8 to 9.0) |
| To use the cohort constructed for the randomised trial in order to explore the association of expressing pattern and lactation outcomes                                                         | No change in any breastmilk at 36 weeks' PMA                                   | Adjusted RR 1.03<br>(0.90 to 1.19)       |
|                                                                                                                                                                                                 | <b>Determinants of day 4 milk quantity (mutually adjusted):</b>                |                                          |
|                                                                                                                                                                                                 | Expressing frequency (per additional session)                                  | 30.5g (15.7 to 45.3)                     |
|                                                                                                                                                                                                 | First expression within 6 hours of birth                                       | 90.1g (24.9 to 155.3)                    |
|                                                                                                                                                                                                 | Electric pump use                                                              | 116.6g<br>(40.9 to 192.3)                |
|                                                                                                                                                                                                 | Simultaneous expressing                                                        | 112.7g<br>(29.6 to 195.7)                |
|                                                                                                                                                                                                 | Prior breastmilk feeding experience                                            | 204.3g<br>(125.2 to 283.3)               |
|                                                                                                                                                                                                 | <b>Determinants of day 21 milk quantity (mutually adjusted):</b>               |                                          |
|                                                                                                                                                                                                 | Expressing frequency (per additional session)                                  | 94.4g<br>(62.7 to 126.2)                 |
|                                                                                                                                                                                                 | Prior breastmilk feeding experience                                            | 335.9g<br>(181.8 to 490.1)               |
|                                                                                                                                                                                                 | <b>Determinants of exclusive breastmilk at 36 weeks' PMA:</b>                  |                                          |
|                                                                                                                                                                                                 | Expressing frequency not associated (per additional session)                   | Day 21 RR 1.05<br>(0.97 to 1.14)         |
|                                                                                                                                                                                                 | 24-hour milk quantity is associated (per 100g)                                 | Day 21 RR 1.06<br>(1.01 to 1.12)         |

CI = confidence interval. SMD = standardised mean difference. MD = mean difference. Med Diff = median difference. RR = relative risk. PMA = post-menstrual age



### **8.3.1 Systematic review**

In Chapter 3, I presented a systematic review of the influence of relaxation interventions on lactation and wellbeing outcomes. The review included eighteen studies conducted in nine countries and data was reported on 1982 participants. Interventions included relaxing music, self-directed guided relaxation/mindfulness and meditation/mindfulness training. Populations ranged from mothers of very preterm infants expressing milk in the NICU to mothers of healthy infants up to a year of age.

There was high certainty evidence that relaxation reduces maternal stress by a small amount, increases infant weight gain by a moderate amount (in the context of direct breastfeeding) and has no impact on milk protein. There was moderate certainty evidence that relaxation increases breastmilk quantity by a moderate amount, reduces maternal anxiety by a small amount, induces a physiological relaxation response, increases milk energy and carbohydrate by a small amount and has no impact on infant length. All other outcomes were classified as low or very low certainty evidence. Key effect estimates are provided in Table 8.1.

One key weakness of the literature as a whole was risk of bias. This was due to poor study quality in some cases but even well-designed studies were challenged by inherent difficulties with blinding and missing data for participant-reported outcomes. The second key weakness of the literature was imprecision, due to the relatively small number of studies and participants for most outcomes.

### **8.3.2 Randomised study**

In Chapter 4, I presented the influence of parent involvement in the planning of the randomised study, whose methods are described in Chapter 5. There were a range of important influences on study design and the modification of the intervention itself (originally designed for mothers of healthy infants) for the target population. The implications of the input gathered from parents extends more widely than simply preparation for this study and included useful insights into mothers' lactation-related objectives and questions to be addressed by future research.

The results of the randomised study in mothers of very and extremely preterm infants were presented in Chapter 6. The study randomised 132 mothers of infants born between 23+0 and 31+6 weeks' PMA in the first three days after birth to provision of a guided relaxation and lactation-specific visualisation recording or standard care, with a request to listen frequently while expressing milk. There was insufficient evidence to demonstrate an effect of the intervention on the primary outcome of expressed milk quantity, or any secondary outcome related to expression efficiency, long-term breastfeeding or maternal anxiety and post-traumatic stress. The majority of participants liked the intervention and reported finding it relaxing. Key effect estimates are provided in Table 8.1.

The effect size for milk quantity reported, while not statistically significant, would have clinical importance and the trial was not powered to detect an increase of this size. Intervention group participants who listened to the recording at higher frequency had a higher milk quantity than those who listened less frequently. Although this was not a randomised comparison, no baseline or expressing behaviour confounders were identified. However, residual confounding cannot be excluded.

A new hypothesis was proposed that relaxation interventions have a smaller effect on milk quantity and mental health when baseline lactation challenge and mental health difficulties are higher. The suggestive evidence for this hypothesis was meta-regression of the effect estimate for milk quantity in relation to mean gestational age in each study, once my RCT was added to meta-analysis. In addition, the direction of the sub-group analysis within the RCT was consistent with this, although not statistically significant (higher effect estimate in mothers of very preterm infants than mothers of extremely preterm infants).

### **8.3.3 Descriptive and exploratory analysis**

Chapter 7 used the data gathered within the randomised study to address other questions surrounding determinants of lactation outcomes after very preterm birth. Regression analysis addressed pre-specified questions using lactation outcomes of exclusive breastmilk provision at 36 weeks' PMA, 24-hour milk yield and hourly milk yield on day 4, 14 and 21 after birth.

Repeated measures of expressing provided a rich descriptive dataset on the trajectories of milk expression parameters. Only a fifth of participants achieved each of the three key clinical recommendations from the UNICEF UK Baby Friendly Initiative; 21% initiated expression within 2 hours of birth; 14-21% expressed eight or more times per day (on day 4, 14 and 21) and 20% expressed at least 750ml in 24 hours at day 14 after birth. This was despite 70% of the cohort planning to exclusively breastmilk feed their infant/s by discharge home, and the fact that 90% of participants were recruited in units with level three BFI accreditation (suggesting a good level of lactation support).

Adherence to the slightly more lenient WHO recommendations was much better, although still suboptimal, with 52% expressing within 6 hours of birth and 28-44% expressing seven or more times per day (on day 4, 14 and 21). Expressed milk yield showed very wide inter-individual variability at all timepoints and less than 50% of this variability could be explained by factors included in analytic modelling.

Long-term breastfeeding outcomes demonstrated a steep drop in exclusive breastfeeding after 36 weeks' post-menstrual age and a very low rate of 14% exclusive breastmilk feeding at four months' corrected age. Maternal anxiety was extremely high in the first three weeks after birth (73% exceeding the threshold for clinical anxiety at baseline and 57% at day 21 after birth). Post-traumatic stress was also evident in a significant minority (18% exceeding the threshold for clinical post-traumatic stress at day 21).

Detailed analyses of the relationships between expressing pattern and lactation outcomes were presented in Chapter 7. Expressing frequently was associated with milk yield, but not long-term exclusive breastmilk feeding. Although expressing eight times or more was clearly superior in relation to milk yield than expressing less than six times a day, evidence related to expressing six to seven times was inconclusive. For many participants, expressing six or more times per day was sufficient to achieve the BFI target yield of 750g (by day 21 after birth).

The longest gap between milk expressions was similarly related to milk yield, in an inverse direction. The specific circadian timing of each expressing session was not associated with hourly milk yield after

adjustment for the gap to prior session. There was a much higher milk yield in mothers who had more than six months of prior breastfeeding experience and therefore these mothers were able to express less frequently. Key effect estimates are provided in Table 8.1.

## 8.4 Strengths and limitations of the programme of work

Table 8.2 outlines the strengths and limitations of the programme of work, by research area. This includes the importance to families and professionals, generalisability of findings, bias, accuracy, methodological quality and utility for clinical practice.

**Table 8.2: Summary of strengths and limitations of each research area**

|                                                             | Systematic review                                                                                                                                                                      | PPI work                                                                                                                                                       | Randomised trial                                                                                                                                                                                                                         | Exploratory analysis                                                                                                                                      |
|-------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Is the work Important to families and professionals?</b> | Yes - multiple prioritisation processes have identified exclusive/any breastmilk and breastmilk expression in prematurity as research priorities, including WHO                        | Yes - PPI processes throughout research are an important way to integrate family priorities into research                                                      | Yes - targeted by PPI work, qualitative literature and co-produced prioritisation processes                                                                                                                                              | Yes - targeted by PPI work, co-produced prioritisation processes and qualitative literature relating to family questions about expressing recommendations |
| <b>Are the results generalisable?</b>                       | Yes – statistical heterogeneity satisfactory for most outcomes. Wide variety of countries and intervention types represented. No subgroup effects found to suggest variation in effect | Yes – very large number of participants involved at early stages. Diverse participants in later stages (ethnic background, age, gestation at birth)            | Yes - diverse participants (ethnic background, deprivation, gestation at birth, education). Multi-centre recruitment. Characteristics of eligible population accurately captured within recruits in most areas                           | As previous column                                                                                                                                        |
|                                                             | No – high statistical heterogeneity for mental health outcomes. Meta-regression suggests possibility of smaller effect with lower gestation                                            | No – low diversity at early stages. Limited diversity in some areas in later stages (education, deprivation). Lack of non-digital opportunities limited access | No – predominantly Southern English units with level 3 BFI accreditation. Participants had high motivation to breastmilk feed, high breastfeeding prevalence. Trial recruits were more likely to be primiparous than eligible population | As previous column                                                                                                                                        |

|                                              | <b>Systematic review</b>                                                                                                      | <b>PPI work</b>                                                                                        | <b>Randomised trial</b>                                                                                                                                                                                                                                                                          | <b>Exploratory analysis</b>                                                                                                                                                             |
|----------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Are the results unbiased?</b>             | Yes - no evidence of publication bias. Some outcomes included were categorised as low risk of bias                            | Yes – wide range of views sought and large numbers of people involved                                  | Yes - no signal of bias in expressing behaviour or mental health self-report. Unbalanced data completeness for primary outcome but sensitivity analysis and adjustment suggest that this did not bias the data. Thorough pre-specified statistical analysis plan                                 | Yes - pre-specified statistical approaches                                                                                                                                              |
|                                              | No – some studies and outcomes categorised as high risk of bias or some concerns                                              | No - may be some bias in participant views represented (unmeasured characteristics/ intersectionality) | No - unblinded intervention brings potential bias. Low data completeness for mental health outcomes increases risk of bias for these outcomes                                                                                                                                                    | No - lower day 4 milk yield for participants with incomplete data suggests some potential bias in who provided data                                                                     |
| <b>Are the results accurate and precise?</b> | Yes – large number of studies and participants included. Several outcomes had pooled effect estimates with adequate precision | Yes – large number of participants in early stages, in depth discussions in later stages               | Yes - risk assessment in place with regular monitoring and mitigation strategies. Limit to multiple comparisons (reduces risk of type 1 error). Independent validation of primary outcome. Quality control checks of data entry and statistical programs were performed                          | Yes - repeated measures and detailed expressing logs increased the power of the dataset                                                                                                 |
|                                              | No – some outcomes had small number of participants and pooled effect estimates did not have adequate precision               | No – little opportunity for participants to challenge interpretations of their responses               | No - Wide confidence intervals, including effect sizes of clinical importance. Underpowered to identify the effect estimate found, and for binary secondary outcomes. Reliance on participant data entry may have caused data inaccuracies. Some evidence of erroneous recording of milk weights | No - statistical power limited by overall sample size and by small numbers adhering to UK expressing recommendations (increases risk of type 2 error). Likely some residual confounding |
| <b>Is the method high quality?</b>           | Yes - high quality methods used, as defined by Cochrane                                                                       | Yes – most best-practice PPI recommendation followed                                                   | Yes - high quality Clinical Trials Unit processes adhered to. Recruitment target achieved. Risk assessment in place with mitigating strategies and regular review                                                                                                                                | Yes – appropriate selection of statistical techniques for questions posed. The high motivation to breastmilk feed made this a good model for study                                      |

|                                                     | Systematic review                                                                                                                                           | PPI work                                                                                                                                                                              | Randomised trial                                                                                                                                                                                                                                                                                                                                                     | Exploratory analysis                                                                                                                                                                                                                                 |
|-----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                     |                                                                                                                                                             | No – some elements of best-practice not followed, such as addressing power imbalances                                                                                                 | No – thesis candidate taking Chief Investigator role and performing most of the recruitment and performing statistical analysis posed some challenges to the usual separation of blinded and unblinded roles (but the CTU was satisfied that these were sufficiently mitigated by involvement of independent statisticians, use of dummy data, validation and so on) | No – nature of exploratory analysis is hypothesis generating but may suffer from post hoc interpretation                                                                                                                                             |
| <b>Are the results useful in clinical practice?</b> | Yes – clinically meaningful outcomes with high and moderate certainty make useful clinical recommendations possible                                         | Yes - via influence on study design and identification of future research areas. Additionally via direct dissemination and publication to the wider clinical and academic communities | Yes - combination with the systematic review findings increases utility by proposing new hypothesis on modifiers to effect size. Qualitative comments are valued by families and professionals                                                                                                                                                                       | Yes – as there is so little data available to inform expressing recommendations, these clinically-focused analyses improve the overall data quality, allowing suggestions for increased nuance of clinical recommendations and hypothesis generating |
|                                                     | No – some outcomes with low and very low certainty could not inform clinical recommendations that families and professionals would have liked to know about | No – indirect method, not a formal qualitative analysis                                                                                                                               | No - limitations to recommendations possible for the specific population due to under powering.                                                                                                                                                                                                                                                                      | No - limitations to strength of potential clinical recommendations due to study size and small number of participants achieving current recommendations                                                                                              |

## 8.5 General implications of the findings

### 8.5.1 Potential mechanisms of relaxation effect on lactation quantity

Three hypotheses were outlined in Chapter 2 for the potential mechanism of relaxation to influence breastmilk quantity. These can be re-examined in light of the evidence obtained in the randomised trial and in the systematic review.

The first hypothesis was that the hormonal changes induced by a relaxation response (for example a reduction in cortisol) could modulate lactogenic hormones (prolactin and oxytocin). The systematic review demonstrated very low certainty evidence that relaxation has no effect on salivary cortisol in lactating women, and low certainty evidence that it reduces breastmilk cortisol. There was also high and moderate certainty evidence for a reduction in stress and anxiety, and the induction of a 'relaxation response' in relation to blood pressure and other vital signs, which could result in unmeasured hormonal changes. There was no data on oxytocin or prolactin concentrations or activity in people provided with relaxation. Some general association of plasma prolactin and oxytocin with anxiety level and salivary cortisol has been seen elsewhere in the literature<sup>115,117</sup>. The evidence gathered and reviewed here provides a small amount of weak evidence that relaxation induces changes in the maternal hormonal environment that could potentially influence lactation hormones.

The second hypothesis was that mental visualisation of milk ejection could enhance or facilitate milk ejection, and that a repeated auditory stimulus could reinforce milk ejection through conditioning. If this were the case, we would expect that women would experience quicker and/or more milk ejections during the expression period and therefore express more milk per minute of expression. However, in the randomised trial we saw no difference in the rate of expression between the two allocation groups. One participant reported milk ejection occurring in response to solely listening to the recording, which has also been reported in one previous study<sup>108</sup>. However, overall the small amount of evidence gathered does not support this hypothesis.

The third hypothesis was that relaxation could change the mother's self-efficacy and lactation-facilitating behaviours. The systematic review demonstrated low certainty evidence for no change in breastfeeding self-efficacy or expressing frequency. In addition, the randomised trial showed no difference in expressing frequency or duration between the two allocation groups. The small amount of evidence gathered does not support this hypothesis.

Overall, none of the hypotheses are strongly supported but evidence is of low certainty so further studies have the potential to change these conclusions. At present, the first hypothesis of hormonal modulation remains the most promising.

### **8.5.2 Patterns of breastmilk feeding after very preterm birth**

The descriptive analyses presented in Chapter 7 showed that both exclusive and any breastmilk feeding prevalence dropped rapidly between 36 weeks' PMA and two months' corrected age (13 weeks later; at 49 weeks' PMA); for example, an absolute decrease of 44% in exclusive breastmilk feeding over this period. Most very preterm infants are discharged home between 36 and 40 weeks' PMA, and most extremely preterm infants are discharged home between 40 and 44 weeks' PMA<sup>286</sup>. The period surrounding discharge is therefore also the period of most rapid drop in breastmilk feeding, particularly exclusive breastmilk.

There are many possible hypotheses for this pattern, including the growing size of the infant (requiring more milk and hitting a ceiling of maternal milk supply), difficulties in transitioning from gavage to oral feeding (particularly direct breastfeeding), and the change in environmental factors between hospital and home (such as parental work patterns, available support and other demands on the parents). There may be motivational influences such as discharge marking a point of reduced fragility for the infant and therefore less perceived need for breastmilk, or a perception of judgement over feeding decisions from hospital staff, as noted in parent responses on the definition of breastfeeding 'success' in Chapter 4.

The trajectory is similar to that seen in National Maternity and Infant Feeding Surveys since 1995<sup>288</sup> if breastmilk feeding at birth in these sources is substituted for 36 weeks' gestation here – i.e. a steep drop in breastmilk feeding in the first six weeks and then a shallower reduction in the following months. Therefore this could reflect sociological influences that are shared by healthy and ex-preterm infants when they get into the home environment, rather than preterm specific factors.



The prevalence of any breastmilk at two months' corrected age in this sample (58%) was lower than that reported at two to three months' corrected age after very preterm birth in China<sup>160</sup> and Sweden<sup>19</sup> (70-72%) but similar to Norway<sup>20</sup> (62%). Similarly, the rate of exclusive breastmilk at two months' CA here (26%) was lower than China<sup>160</sup> and Sweden<sup>19</sup> (33-44%). This is as expected given national variation in breastfeeding outcomes, which are correlated between term and very preterm infants; i.e. countries with higher national breastfeeding rates also have higher rates after very preterm birth<sup>65</sup>.

In a similar pattern, the rates of any and exclusive breastmilk at four months' corrected age (46% and 17% respectively) were lower than those reported in Sweden<sup>19</sup> (55% and 34% for very preterm infants and 56% and 26% for extremely preterm infants respectively). As the infants in our sample were an average of 6.7 months actual age at this timepoint, they can also be compared with a number of studies reporting feeding outcomes at six months of actual age. Any breastmilk at six months of actual age in very preterm infants in the literature varies from a low of 27% in Portugal<sup>301</sup> to a high of 53% in Sweden<sup>302</sup>. Again, this is similar to the variability in background breastmilk feeding rates in different countries and regions, so is consistent with existing research paradigms.

## **8.6 Clinical and policy implications**

### **8.6.1 Relaxation and lactation in the NICU**

The GRADE approach provides thorough guidance<sup>303</sup> on moving from the assessment of evidence to a clinical recommendation, making it clear that the decision on making a weak (conditional) or strong recommendation depends not only on the strength of the evidence for and magnitude of benefit/harm but also on the values and preferences of patients, and issues of healthcare resource. 'Weak' and 'strong' will be used in the rest of the chapter to denote these GRADE-specific definitions.

The greatest theoretical potential benefits that mothers of very preterm infants could gain in using relaxation interventions would be a positive cognitive experience (for example, perception of relaxation and liking the experience), improved mental health, increased expressed milk quantity and increased milk

protein (as this is the key macronutrient that is insufficient for the very high growth rates of very preterm infants within breastmilk).

Of these, the systematic review provided moderate and high quality evidence of benefit in milk quantity, anxiety and stress. Our trial reported benefit for some participants in perception/experience. However, our trial results suggested a hypothesis that the mental health and milk quantity benefits of relaxation are reduced (or eliminated) for mothers with high levels of anxiety and distress, and with a high level of lactation challenge.

The potential harms to mothers of very preterm infants in using relaxation interventions are a negative cognitive experience (for example, perception of feeling less relaxed, disliking the experience or feeling pressured to listen), worsened mental health and the time burden/increased mental load of an additional task attached to milk expression.

Apart from general mental health measures (which showed no negative effects), potential harms were only measured in my study and therefore it can only be concluded that there was evidence of minor harm to some participants in their cognitive experience and perception of relaxation with this specific relaxation intervention. This harm could potentially be mitigated by providing a variety of relaxation resources, including relaxing music.

There is little evidence available to judge the values and preferences of mothers of very preterm infants in relation to the use of relaxation interventions. Relaxation is a well-received intervention that is predominantly seen positively by the general public<sup>101</sup>. It was clear that some participants highly valued the intervention regardless of any influence on milk expression. However, some mothers felt that the intervention was not worth the burden imposed; this was suggested in participant comments and also in the imbalance in loss to follow up (with intervention participants more likely to disengage) and over-representation of primiparous individuals compared to the eligible population.

Relaxation interventions do not have a major impact on healthcare resources. Relaxing music is particularly easy to recommend to parents without any resource transfer being required as it is almost universally available. The intervention used in this study is now freely available and so can be used with minimal cost by any mother with an audio device and an internet connection. However, there is a resource implication for mothers themselves due to the additional time required to integrate relaxation into the expressing routine.

Considering all of these domains of GRADE guidance, a weak recommendation can be made to make relaxation interventions available to mothers of very preterm infants, without any pressure to pursue relaxation if it is not in line with the mother's individual values and preferences. Mothers of very preterm infants can be advised that many people enjoy and value relaxation in this setting and that there may be a small increase in expressed milk quantity. If they do not enjoy using relaxation, then it is unlikely that the magnitude of beneficial effect would outweigh the time burden and negative experience.

For lactating mothers as a general population, the same recommendation can be made, with more confidence in the potential benefit of relaxation. These mothers can be advised that relaxation is likely to cause a small or moderate reduction in stress and anxiety and a small or moderate increase in milk quantity and infant weight gain.

## **8.6.2 Recommendations on how to express milk in the NICU**

### **8.6.2.1 *A useful analogy***

The implication of our findings for clinical recommendations on how to express milk are more complex because of the lower strength of evidence and conflicting potential reasoning approaches to justify decision-making. To facilitate the discussion of the underlying tensions in attempting to derive these recommendations, I will first outline an analogous area that has been the topic of a large volume of debate and literature; this is the age of introduction to complementary food (CF, "solids" or weaning) in the general population.

Prior to 2001, the World Health Organisation and most national bodies recommended the introduction of CF at 4-6 months of age, but for WHO this changed to 6 months of age after a systematic review<sup>304</sup>. The most up to date version of this systematic review was published by the WHO in 2023<sup>305</sup>. In relation to the comparison of introducing CF before or at/after six months of age, it noted low and very low certainty evidence that earlier introduction may increase overweight, and may reduce iron deficiency anaemia (i.e. potential harm and potential benefit). There was no evidence for an impact on infections or atopy. No potential benefit was identified for introduction of CF before 4 months of age, in comparison to at/after six months.

Using this systematic review, the WHO gave a strong recommendation that "infants should be introduced to complementary foods at 6 months (180 days) while continuing to breastfeed"<sup>306</sup>. There is an additional element of nuance, stating "the recommendation is a public health recommendation and recognizes that some infants may benefit from earlier introduction of complementary foods". A strong recommendation indicates that the guideline development group (GDG) "was confident that the desirable effects of adherence to the recommendation outweigh any undesirable consequences".

Further detail is given on the reasoning behind a strong recommendation despite low certainty evidence: "there were small but uncertain evidence of benefits from introducing complementary foods at 6 months compared to earlier... on balance, the balance of benefits and harms favoured introduction at 6 months rather than earlier... Introduction of complementary foods before 6 months compared to at 6 months would be more costly because of the cost of the quality and variety of foods to optimally feed infants. However, introduction at 6 months could also be costly for women who are exclusively breastfeeding if they lack a supportive environment and must forgo paid work."

A different emphasis was placed by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) after an evidence review up to 2016<sup>307</sup>, stating that for European infants "complementary foods... should not be introduced before 4 months but should not be delayed beyond 6 months." The European Food Safety Authority (EFSA) made a similar conclusion, stating "it is likely that

there is an appropriate age range rather than a single appropriate age for the introduction of CFs... The available data do not allow the determination of a single age for the introduction of CFs for infants living in Europe. The appropriate age range depends on the individual's characteristics and development"<sup>308</sup>.

ESPGHAN<sup>307</sup> notes that infants who are exclusively breastfed may have a different balance of harm and benefit related to CF timing than those receiving some or exclusive infant formula as their milk diet, and that evidence relating to exclusively breastfed infants cannot be extrapolated to those not receiving the specific benefits of breastmilk in this way. ESPGHAN<sup>307</sup> also note that societal influences may alter the balance point, for example the availability of clean water supplies and the overall likelihood of overweight versus stunting.

Some authors propose that recommendations should be made separately for subgroups of infants such as those fed infant formula<sup>309</sup>. Others have proposed that recommendations should be entirely personalised, guided by signs of motor developmental readiness<sup>310</sup>. One notable 'rapid response' to a 2011 commentary<sup>311</sup> made an argument to move the locus of decision-making to the family, saying that "mothers need to be able to weigh... drawbacks against the benefits... Let's be honest about what the evidence actually does show in terms of benefit or lack thereof, and let parents make a fully informed choice for themselves as to whether or not they find those two extra months of avoiding solids... worthwhile."

A final important point for our analogy is that adherence to WHO CF recommendations is relatively low in practice. Introduction of CF below six months of age takes place in between 19% (South Asia) and 48% (Latin America) of infants worldwide<sup>306</sup>; in the UK in 2020, 40% introduced CF by five months of age<sup>228</sup>. However there is evidence that the change in guidance from 4 to 6 months has shifted behaviour, with UK national surveys showing a reduction in CF before 4 months of age from 45% in 2005 to 14% in 2020<sup>228</sup>. Therefore even if the ESPGHAN recommendation of CF introduction between 4 and 6 months is accepted, it could be proposed that messaging to wait until six months is more effective to achieve population adherence of this goal. However, the potential for stress and guilt for those who feel they should wait

until six months but don't, and the potential loss of trust in health professional advice, need to be weighed against such reasoning.

This analogous debate has drawn out the following points that will be useful to the subsequent discussion of expressing recommendations. These are that:

- Different groups of experts may form different behaviour recommendations where evidence is low certainty, because the size of the potential benefit/harm is weighed against other values such as costs and the specific context (for example, global versus European)
- Some approaches place more value on informing individuals of specific evidence rather than seeking to recommend a general approach
- There is conflict between creating a simple and universal message (a public health recommendation) versus individualised care, taking into account specific risk factors
- A more extreme recommendation may leverage a greater shift in the population distribution of behaviour. However there are risks of this approach in causing harm to those who find it difficult to achieve the recommendation or 'fail' to adhere; and in compromising trust

#### 8.6.2.2 Reasoning for expressing recommendations

As noted in the introduction, UNICEF BFI summarises their expressing pattern recommendation and evidence base in this way:

*"In order to maximise a mother's potential to provide milk for her sick or preterm infant, it is essential to try to mimic what would happen if she had her 'term' baby with her from birth... mothers of preterm babies should be encouraged to... express a minimum of eight times in 24 hours, including once at night time... There is not a great deal of empirical evidence to suggest frequency of expression."*<sup>24</sup>

This is an example of both mechanistic and precautionary principle reasoning<sup>312</sup>. Mechanistic reasoning makes an inference based on a physiological rationale; in this case the implicit rationale that evolution has shaped infant behaviour and milk supply in tandem and therefore mimicking the typical pattern of breast stimulation is likely to be optimal. The precautionary principle values the most conservative

approach in a situation of uncertainty; in this case setting a high bar for expressing frequency in the face of minimal evidence and the knowledge that lactation outcomes are often poor after very preterm birth.

Using mechanistic and precautionary principle reasoning as a basis for clinical guidance is often seen as useful only if frequentist (classical “evidence-based”) reasoning<sup>312</sup> is not possible. In this context, frequentist reasoning would derive recommendations from evidence of association of particular expressing patterns with desirable outcomes in the specific population of mothers expressing milk after very preterm birth, or interventional studies examining different approaches. If insufficient evidence is available then frequentist reasoning would result in no recommendation, or a weak recommendation.

If we accept that the frequentist approach is optimal where it is possible, and referring again to the GRADE approach<sup>303</sup>, it is necessary to review the strength of evidence for and magnitude of benefit/harm of different expressing patterns, the likely values and preferences of patients, and issues of healthcare resource.

The systematic search described in the introduction to Chapter 7 found that the small number of previous studies support the hypothesis that expressing frequently (defined as at least six to seven times per day) is beneficial for milk quantity<sup>36,39,40,273</sup>. My own analysis is the only attempt to examine expressing more than eight times a day specifically and did not find conclusive evidence that this is significantly better than expressing 6-7 times a day in terms of milk quantity. There is conflicting evidence on whether expressing frequency is associated with long term breastfeeding; when including my own analysis, most studies conclude that these two factors are not associated<sup>38,276,277</sup>, although there are more participants in the studies that do show an association<sup>31,134,153</sup>.

The evidence related to the longest gap between expressions is even weaker as it relies solely on my own analysis and one additional study<sup>31</sup>, which were conflicting. My own analysis is the only attempt to examine the circadian timing of expressions, finding no evidence that night-time hours are associated with higher milk yield.

Given that expressing frequency explained only 10-20% of the variability in milk yield in this study, and milk expression quantities consistently show very high variability in the literature<sup>36,40,283</sup>, the likelihood of a single recommendation being optimal for all is low. Even when evidence is of high quality, applying population data to individuals is philosophically problematic: “taking a population or sub-population approach and looking at average results in these populations opens up a gap between the empirical evidence generated... and the needs of individual patients”<sup>313</sup>.

The evidence-based medicine paradigm treats individuals like the roll of a dice; an evidence-based recommendation will not be optimal for every patient but with high quality evidence it should be optimal for a high proportion of them<sup>312</sup>. These philosophical difficulties are exacerbated where individual variability is high and evidence quality is low, as in our case; the recommendation to express eight or more times a day is likely to be suboptimal for many parents. As in the analogous case of complementary feeding, a global recommendation seeks to optimise population behaviour; at an individual level it can only be used as a springboard for individual decision-making.

The qualitative literature surrounding the experience of expressing milk after very preterm birth supports the premise that most mothers dislike expressing frequently, with short gaps and with interrupted sleep, but most mothers also value increased milk yield<sup>11,14,26,29,75,92</sup>. There is likely to be a variable risk/benefit assessment at an individual level between the negative experience of expressing frequently versus a positive impact of increased milk yield. There are few implications of expressing pattern recommendations on healthcare resources (although more breast pumps and equipment may be required), but there are large implications on the resources of individual families, in terms of time and opportunity cost. Supporting families to actually express at high frequency, rather than just recommending that they do so, could have greater impact on healthcare resources as this might require subsidy of travel and childcare for other children, for example.

If no current clinical recommendations existed and using the GRADE approach, these factors would not support a strong recommendation to express eight or more times a day. A more appropriate approach



might be a weak recommendation to express frequently (for example, more than 6-7 times per day) with gaps no longer than six hours.

The current UNICEF BFI recommendations are widely known by UK professionals supporting families, and by many families of very preterm infants. Families strongly value consistency of messaging, and consistency is felt to be an important aspect of effective public health communication<sup>314</sup>. A relatively minor change in recommendations (from 8 or more times per day to 6-7 or more times per day), based on relatively weak evidence may not be considered worth the risk of creating inconsistent messaging. However, UNICEF BFI have changed their recommendations before based on weak evidence, for example moving from a recommendation to express within six hours of birth if breastfeeding is not possible to expressing within two hours of birth. In addition, the WHO recommend expressing 7 or more times per day, so a change in guidance may create higher consistency internationally.

In the current situation, where families are recommended to express eight or more times a day, the actual expression frequency is generally six times a day or less, as seen in this study and elsewhere in the literature<sup>32-34,40,281</sup>. There is some anxiety among professionals that decreasing the recommendation would result in a further decrease in actual expression frequency (analogous to the discussion of complementary feeding above). However, consciously providing a recommendation that is known to be over-exaggerated in order to manipulate parents' behaviour poses ethical difficulties. The ethical difficulties are two-fold; firstly that honesty is a foundational value to build trust between professionals and patients, and secondly because setting recommendations that are difficult to achieve without justification is likely to cause harm to mothers by creating stress and guilt.

The literature surrounding public health messaging in the SARS-CoV-2 pandemic provides a useful paradigm to consider whether it is ethical to use clinical guidance to persuade rather than simply inform<sup>315</sup>. As a baseline, it is acknowledged that all guidance should start with an assumption of honesty, outlining benefits and risks to patients with an acknowledgement of the evidence quality. This respects the rights of the individual patient and enables informed decision making, as well as fostering trust<sup>315</sup>. It

is an established basis of behaviour change support to provide information and support the individual to weigh these up in order to foster self-efficacy and personal commitment to the desired behaviour change<sup>316</sup>.

However, it is acknowledged that this approach may not maximise adherence because many people prefer simple, concrete messages and because it relies on the patient having sufficient capacity to take in and weigh up more complex information. This could result in inequities based on patient background<sup>315</sup>, particularly marginalising factors such as English proficiency, learning disability and lower levels of education.

Alternative approaches move along a spectrum from informing to recommending, persuading, manipulating and, in the extreme case, coercing. Recommending/persuading paradigms would emphasise a simple concrete action (in this case to express eight or more times a day) while ignoring the disadvantages and the uncertainty over evidence. This is a form of exaggeration or 'spin'<sup>315</sup> and I would argue that the current UK clinical recommendation does fall into this category, albeit unintentionally.

Some authors suggest that a persuasive approach is only justified where the likely pros for the individual far outweigh the likely cons, and/or the adherence of one individual has a significant impact on wider society (as is the case for infectious disease)<sup>315</sup>. Our case does not fall into either category, so a persuasive approach is unlikely to be ethically justified.

The analogous complementary feeding setting consciously moves beyond the informing approach because of its acknowledged function as a public health recommendation; however it attempts to ameliorate this with a full description of the strength of the evidence, the reasoning of the guideline group decision and the statement that the recommendation may not be optimal in every case. These latter aspects would be useful to add to the UNICEF expressing recommendations to increase legitimacy.

There is some evidence from qualitative literature presented in Chapter 2, and in our parent involvement process presented in Chapter 4, that the recommendation to express eight or more times a day is seen by

many as unrealistic. This can induce distress and a feeling of hopelessness at the impossibility of achieving the target and may lead to complete cessation of expressing<sup>11,14,26,29–31,75,92</sup>. Public health literature notes that a persuasive approach can lead to stigma and shame if the recommended action or result cannot be achieved<sup>315</sup>. In addition where a message is not perceived to chime with the person's current beliefs and expectations, it can actually decrease its acceptance compared to no messaging at all<sup>317</sup>. For example, in our analogous complementary feeding debate, some authors have argued that in the setting of very low rates of adherence to guidance, a more extreme guideline might be counter-productive as the distance between reality and the ideal is just too great<sup>318</sup>.

In general, advising less 'drastic' behaviour change is thought to lead to less negative/oppositional reaction by the public<sup>319</sup>. In addition, language that is forceful is less effective than language that focuses on suggestion and empathy<sup>319</sup>. All of these factors cast doubt on the efficacy of a strong recommendation to express eight or more times a day, and on the concern that reducing the recommendation would cause parents to further reduce their expressing frequency.

The reality of adherence to clinical advice is based on what a patient finds resonant, culturally congruent and affordable<sup>312</sup> (here interpreting affordability predominantly in terms of time allocation). If the clinical recommendation for very frequent expressing is not found to be resonant and affordable for most people, then it will not be perceived as 'true', is not pragmatic and may even be counter-productive.

Ethnographic work has shown that professionals who set healthcare guidance do consider how realistic or usable a recommendation is, as well as the quality of the evidence behind it<sup>320</sup>. However a recommendation that is unrealistic may be useful in order to drive change. For example in the formation of a hypertension guideline, a recommendation for low salt intake was felt to be unrealistic for those experiencing poverty given that current low-cost food options tend to be high in salt. The recommendation was retained with acknowledgement that it might leverage political change to regulate the food industry.

In relation to our topic, this idea might translate as a recommendation for very frequent expressing driving enhanced support to families to reduce other burdens to facilitate the achievement of this difficult task. That is, instead of following the general feeling that frequent expressing is unrealistic, to focus on helping families to make it more realistic. However, we have noted that the strength of the evidence for expressing at the perceived unrealistic frequency of eight or more times a day is low so this is not a compelling argument.

How can we integrate these various philosophical and practical arguments? My suggestion would be to move to a recommendation to express at least 6-7 times a day, with gaps no longer than 6 hours, and add that this may not apply to all individuals. However, if UNICEF BFI did not feel that change was worth the loss of consistency and need for retraining, then I would suggest retaining the established simplistic message to express eight or more times a day, but noting that this may not apply to all individuals and making more information available to families on the evidence uncertainties in order to support individualised decision-making.

In practice, neonatal nurses, doctors and midwives without a specialist interest in lactation or specific time allocated to lactation support can use the simplistic 'rule' or cue<sup>313</sup> (either 6-7 times, or eight times per day) in order to provide brief support. The health psychology literature would also support a slight change in emphasis for increased efficacy. Instead of telling people that they 'must' or 'should' express eight or more times a day, messages such as 'try to' and 'you might want to' may be more effective<sup>319</sup>.

Lactation specialists with more time for nuanced discussions with families and increased continuity of care could take the 'rule' as a starting point and use evidence to support families to judge whether they want to move to a more personalised target based on their risk profile as well as their values and circumstances<sup>313</sup>. This is an example of 'tinkering' reasoning<sup>312</sup> – starting where the patient is and making small changes sequentially over time, which is useful when evidence is weak. It also exemplifies a suggested approach to put in place different guidelines for professionals with different levels of

experience<sup>312</sup>, and a suggested balance point of providing simple, actionable messages for all while making more detailed information available for those who want to explore further<sup>315</sup>.

This study alone does not provide sufficient evidence to support personalised decision-making a priori but could be used as a guide for these small changes with examination of subsequent milk yield. For example, supporting mothers with good milk yield to slowly reduce expressing frequency if desired, particularly if they have previous breastmilk feeding experience.

In conclusion, although there is adequate evidence that expressing frequently is beneficial for lactation, the strength of the evidence does not align with the current strength and focus of UK clinical recommendations. There is no clear answer on whether or not to modify the well-known recommendations at this time. However, disseminating the results of this study to lactation professionals (for example, infant feeding leads and nutrition specialist doctors and neonatal nurses) and to the public, could support individualised discussions with families to improve their decision making around expressing patterns in the context of their values and life circumstances.

In practice, until UNICEF BFI choose to amend their recommendations, dissemination material of the results of this study will need to include the current recommendation in order to reassure neonatal unit staff that using the material will not compromise their BFI accreditation. This is reflected in the social media material provided in Appendix K.

## **8.7 Implications for future research**

### **8.7.1 Relaxation therapy for lactating mothers**

The systematic review demonstrated that one key problem for the certainty of conclusions was imprecision. Therefore more randomised studies of relaxation and lactation would be likely to increase certainty, and may give insights into reasons for heterogeneity among results; i.e. are some types of relaxation intervention/dose/settings different to others in their effects? Future studies should ensure high quality trial processes to minimise bias through study design, which has been a problem with many

of the currently published studies. The issue of dose response effects would be a useful area for parents to know more about, with current evidence at risk of confounding, so randomising parents to different recommended doses of relaxation within a future trial would be useful.

It could be argued that further investment of financial resources in randomised trials of relaxation and lactation is not the most efficient use of those resources. Given the low risk of harm of relaxation interventions and subjective benefit outside of the lactation domain, parents may feel that the current level of evidence is sufficient for decision making. However, it is likely that the population most in need of a higher level of certainty are those who are exposed to most stress and who are most time-poor; for example, mothers of very preterm and sick infants in the NICU. Given that we have hypothesised that these mothers will experience the least benefit from relaxation, further research in this specific population is justified.

An alternative reason to continue interventional research related to relaxation would be to further examine the causal pathways of an effect on lactation. As I discussed within the section on general implications above, current evidence for causal hypotheses is scant and unconvincing. Insights gained into mechanism could highlight alternative approaches to improve lactation outcomes, or suggest ways of targeting relaxation strategies to specific populations. In addition, qualitative research on the barriers and facilitators of the use of relaxation after very preterm birth could be helpful for implementation.

### **8.7.2 Clinical recommendations on expressing milk**

I have outlined the ethical difficulties of the current recommendations for milk expressing pattern and the need for more personalised recommendations in the context of high inter-individual variability. This will involve large scale observational evidence of a diverse group of mothers and measurement of a very wide range of potential confounders and contributory variables. Particular focus on accurate measurement of prior breastfeeding experience, pregnancy comorbidities and markers of placental or hormonal function is likely to be informative.

Interventional trials are possible comparing different clinical recommendations, but this is likely to require a very large sample size if the change in advice is subtle (for example, comparing a recommendation for expressing eight times or more to a recommendation for expressing six times or more). There are two possible pathways of individualised guidance that could be pursued; firstly attempting to derive a set of recommendations based on known pre-birth risk factors, and secondly starting with a universal recommendation and then ‘tinkering’ according to early lactation outcomes (for example, day 4 milk yield). Qualitative research may have a role in examining parents’ preferences for these approaches, as well as potential barriers and facilitators to behaviour change and perception of resulting stigma or judgement.

There are many other aspects of expressing recommendations that require this higher level of evidence in addition to expressing pattern, as mentioned by parents in Chapter 4. For example, the optimal time to first expression attempt, the optimal mode of expression before and after secretory activation, meaningful milk yield targets over time and how best to clean equipment and store milk. Another avenue of research could address how professionals can better support families to practically achieve recommendations, given low current adherence.

Further novel interventional research to improve milk yield and breastfeeding outcomes is vital and there are several promising non-pharmacological avenues for future randomised trials, such as antenatal expression, breast warming and “hands-on pumping”. There have been a small number of randomised trials of antenatal expression at 36 week’s PMA or later<sup>321</sup>, with an emphasis on safety (whether this precipitates birth). Secondary outcomes in these trials suggest there may be an improvement in lactation outcomes. However no studies have examined the impact of antenatal expression when preterm birth is predicted to occur shortly or is planned for medical indications.

There has been one randomised study of warming the breast prior to milk expression with increased milk yield demonstrated from a warmed breast<sup>322</sup>. “Hands-on pumping” is felt to be a promising technique due to one observational study<sup>273</sup> (without a control group), demonstrating that participants taught this

technique continued to increase their milk yield for many weeks and had a higher milk yield than generally seen in the literature after very preterm birth. This technique is a complex intervention, involving breast massage and compression during the process of expression, targeting increased milk jets, and also advice to hand express after milk flow has ceased with the electric pump.

My results demonstrated a steep drop in breastfeeding rates around discharge home and this area would benefit from more qualitative research to identify potential mechanisms and therefore suggest ways that support can be improved. Documenting normal feeding-related behaviour after very preterm birth in large cohorts and using Deuterium isotope to investigate how much exclusively breastfeeding infants drink from the breast in the months after discharge could improve counselling for families even if potential interventions cannot be identified. For example, understanding normal sleeping patterns, feeding frequency and average milk intake after NICU discharge could allay parental concern over milk supply.

Families raised a variety of issues around the transition to direct breastfeeding in Chapter 4 that are priorities for them in terms of unanswered questions. For example, how to optimise positioning and attachment for small and easily fatigued infants, the role of technology such as nipple shields, supplemental nursing systems, pacifiers, cups and bottles, and how to make decisions around tube and oral feeds. They also noted the importance of studying maternal outcomes such as over-supply, mastitis and blocked ducts, which are often neglected in favour of milk quantity and breastmilk provision to the infant.

In all of these areas of lactation-focused research, future work should be guided by the insights given by parents in Chapter 4. This includes the use of meaningful outcome definitions of lactation ‘success’ and the integration of personalised outcome assessments such as asking for personal goals and exploring parents’ experiences of their lactation support and journey. Increased integration of qualitative experiences into interventional trials would be helpful.

The magnitude of mental health burden experienced by the participants in our trial demonstrated the need for further research in this area, whether or not this is related to lactation. Parents particularly felt



that post-traumatic stress disorder is an under-researched area of postnatal mental health, as noted in Chapter 4.

## **8.8 Lessons learned to apply to future work**

There were a variety of lessons learnt from the programme of work that can be applied to future research, which are summarised here.

### **8.8.1 Parent involvement dynamics**

For future work, PPI collaboration should begin at the very start of the research process, when setting broad questions and methods. PPI collaborators should be empowered and offered training to enable a true partnership rather than a consultation approach. PPI methods should be diverse, using in person contact either directly or via trusted organisations, to overcome digital poverty and communication barriers posed by written, digital formats.

### **8.8.2 Study Design**

For future work, the balance of parent and professional measurement of milk quantity needs careful consideration. Using professionals to measure milk quantity has the potential to increase data accuracy and data completeness. However no barriers should be put in place that might prevent expressed milk from being given directly to infants where this is the optimal clinical process. In addition, consideration of minimising pressure or judgement on parents from the involvement of a professional in milk measurement is needed. Alternative lactation outcome measures such as quantity of mother's milk given to the infant and long-term breastmilk feeding may be helpful, although measurement of milk quantity is likely to be required as a secondary outcome to assess the mechanism of any improvement in breastfeeding prevalence seen.

It is likely that if parent measurement of milk quantity were used in a future trial, using milk volume rather than weight would be most appropriate. This is because using milk weight was difficult to explain to

parents and there were clear errors due to misunderstanding of how to account for the weight of the milk container when reporting results. In addition, the process of weighing milk rather than reading the volume from the container was more time consuming for parents and required them to have the weighing scale with them at each expressing session. If milk volume measurements were used, several other challenges would need consideration. Firstly, how to accurately measure very small quantities of colostrum or breastmilk, either in a syringe (where colostrum can show clumping) or in a bottle (where the milk quantity may be below the lowest volume marking). Secondly, how to account for large volumes of breastmilk, where the quantity may be above the highest volume marking or several consecutive containers may be used. And thirdly, the use of non-neonatal unit expressing containers, where volume measures may be less accurate or not present.

A further learning point related to thorough analysis of expressing dynamics is the impact of single day logs versus daily logs over a specified time period. Single day logs limit the recording of the gap to prior expressing session for the first logged session, which in our study was shown to have different characteristics to other sessions and was influential in analysis results. If daily logs are felt to be too burdensome for parents, an alternative would be to ask participants to note the time of the previous session at the start of the log, or to ask parents to start logging at midday rather than the morning, or for 48 hour stretches rather than 24 hours.

A final learning point on the practicalities of logging milk expression was to account for more potential variations of how people express if these are to be captured accurately. For example, the case report form had a space for milk weight to be recorded for both left and right breast. However, in practice some participants expressed both breasts sequentially into the same bottle and therefore made only one entry on the log. Those who expressed both breasts sequentially might have made two entries in the log with one timing – it would therefore be unclear how long each breast had been expressed. If data on the exact expressing pattern is needed (for example, to include simultaneous expression as a confounding variable), then more thought and PPI input will be required to ensure participant logs are clear enough to elicit this

data accurately. If exact data is not required then it may be better to ask participants to combine milk into one container for measurement, or to provide unlimited space to log any number of containers associated with one session.

### **8.8.3 Intervention modification**

There were several important learning points about the nature of the intervention recording itself. Although the parent involvement process attempted to make the wording more appropriate for parents after very preterm birth while retaining the affirmative and connection-focused narrative, some participants still felt unhappy with this balance. Two participants mentioned feeling sad when the recording talked about imagining contact with the baby, because they couldn't currently touch the baby; one even said it felt 'cruel' to listen to this part. However, both of these participants said they got used to the recording, came to like it and continued listening. This topic would need careful thought and more parent involvement to decide on further modifications.

Further preferences expressed by some participants were for variety in the relaxation resources provided and for longer material duration. Although this was expressed by less than 10% of the intervention group, allowing participants some choice in the selection of relaxation material is generally acknowledged to be useful<sup>108,261</sup> and could have mitigated the harm to participants who reported disliking the intervention. Future trials should also consider putting relaxing music behind a verbal protocol as this was the preference of several participants. However, one previous RCT found lower milk yield when music was added behind the verbal protocol compared to words alone<sup>203</sup> so this may be an area where perception is not in line with physiological effects.

## **8.9 Overall Conclusion**

This chapter has summarised the findings of the thesis research, its strengths and limitations, the clinical implications and the direction that future work should take. The programme of work has provided an in-

depth understanding of two aspects of lactation after very preterm birth; the influence of relaxation and the influence of expressing pattern.

There are two key clinical recommendations for mothers of very preterm infants expressing breastmilk. Firstly, a weak recommendation to use relaxation interventions with advice that many people value relaxation in this setting and that there may be a small increase in expressed milk quantity. Further trials in settings of high lactation challenge, with multiple dose arms and a range of available relaxation materials, would help strengthen the evidence in this time-poor population.

Secondly, a two-pronged recommendation related to expressing pattern. As a clear and consistent message, the advice to try and express eight or more times in 24 hours, including once at night can be retained (or could be modified to 6-7 times in line with WHO advice). However, lactation specialists can use the evidence presented here to support individual families to make personalised decisions around expressing based on their own risk profile, circumstances and values, which may involve a reduced expressing frequency. A significant body of future research is required to better define risk profiles for low milk supply, targeted guidance on optimal expressing and randomised trials of potential interventions to improve expressed milk volume and breastfeeding outcome after very preterm birth, and in other NICU settings.

Finally, findings from the programme of research point to other areas of research that are important to families in lactation and prematurity. These are the transition from expressing to breastfeeding, determinants of post-discharge breastfeeding decisions and normal breastfeeding-related infant behaviour after discharge.

In conclusion, the findings of this thesis will help to improve the clinical support that parents of very preterm infants receive in trying to optimise their lactation outcomes.

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## Appendices

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### Appendix A: Tables and documents supporting the systematic review of lactation and relaxation (related to Chapter 3)

**Table A.1: Line by line search strategies for each database**

**AMED:**

|     |                                                                      |
|-----|----------------------------------------------------------------------|
| 1.  | exp breast milk/                                                     |
| 2.  | exp breast feeding/                                                  |
| 3.  | exp lactation/ or exp lactation disorder/                            |
| 4.  | breastmilk.mp. [mp=abstract, heading words, title]                   |
| 5.  | "human milk".mp. [mp=abstract, heading words, title]                 |
| 6.  | breastfed.mp. [mp=abstract, heading words, title]                    |
| 7.  | breastfeeding.mp. [mp=abstract, heading words, title]                |
| 8.  | "breast feeding".mp. [mp=abstract, heading words, title]             |
| 9.  | "breast milk".mp. [mp=abstract, heading words, title]                |
| 10. | lactation.mp. [mp=abstract, heading words, title]                    |
| 11. | exp relaxation training/                                             |
| 12. | exp guided imagery/ or exp imagery/                                  |
| 13. | exp meditation/                                                      |
| 14. | exp music therapy/                                                   |
| 15. | exp Milk, Human/                                                     |
| 16. | exp Relaxation Therapy/                                              |
| 17. | exp Imagery, Psychotherapy/                                          |
| 18. | exp Relaxation/ or exp Muscle Relaxation/                            |
| 19. | "relaxation therapy".mp. [mp=abstract, heading words, title]         |
| 20. | meditation.mp. [mp=abstract, heading words, title]                   |
| 21. | "guided imagery".mp. [mp=abstract, heading words, title]             |
| 22. | "verbal protocol".mp. [mp=abstract, heading words, title]            |
| 23. | "music therapy".mp. [mp=abstract, heading words, title]              |
| 24. | 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 15                |
| 25. | 11 or 12 or 13 or 14 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 |
| 26. | 24 and 25                                                            |
| 27. | exp music/                                                           |
| 28. | 25 or 27                                                             |
| 29. | 24 and 28                                                            |

**Medline:**

|     |                                                                                                                                                              |
|-----|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1.  | exp breast milk/                                                                                                                                             |
| 2.  | exp breast feeding/                                                                                                                                          |
| 3.  | exp lactation/ or exp lactation disorder/                                                                                                                    |
| 4.  | breastmilk.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word]           |
| 5.  | "human milk".mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word]         |
| 6.  | breastfed.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word]            |
| 7.  | breastfeeding.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word]        |
| 8.  | "breast feeding".mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word]     |
| 9.  | "breast milk".mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word]        |
| 10. | lactation.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word]            |
| 11. | exp relaxation training/                                                                                                                                     |
| 12. | exp guided imagery/ or exp imagery/                                                                                                                          |
| 13. | exp meditation/                                                                                                                                              |
| 14. | exp music therapy/                                                                                                                                           |
| 15. | exp Milk, Human/                                                                                                                                             |
| 16. | exp Relaxation Therapy/                                                                                                                                      |
| 17. | exp Imagery, Psychotherapy/                                                                                                                                  |
| 18. | exp Relaxation/ or exp Muscle Relaxation/                                                                                                                    |
| 19. | "relaxation therapy".mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word] |
| 20. | meditation.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word]           |
| 21. | "guided imagery".mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word]     |
| 22. | "verbal protocol".mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word]    |
| 23. | "music therapy".mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word]      |
| 24. | 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 15                                                                                                        |
| 25. | 11 or 12 or 13 or 14 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23                                                                                         |
| 26. | 24 and 25                                                                                                                                                    |
| 27. | exp music/                                                                                                                                                   |
| 28. | 25 or 27                                                                                                                                                     |
| 29. | 24 and 28                                                                                                                                                    |

**Embase:**

|     |                                                                                                                                                                                                                 |
|-----|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1.  | exp breast milk/                                                                                                                                                                                                |
| 2.  | exp breast feeding/                                                                                                                                                                                             |
| 3.  | exp lactation/ or exp lactation disorder/                                                                                                                                                                       |
| 4.  | breastmilk.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]           |
| 5.  | "human milk".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]         |
| 6.  | breastfed.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]            |
| 7.  | breastfeeding.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]        |
| 8.  | "breast feeding".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]     |
| 9.  | "breast milk".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]        |
| 10. | lactation.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]            |
| 11. | exp relaxation training/                                                                                                                                                                                        |
| 12. | exp guided imagery/ or exp imagery/                                                                                                                                                                             |
| 13. | exp meditation/                                                                                                                                                                                                 |
| 14. | exp music therapy/                                                                                                                                                                                              |
| 15. | exp Milk, Human/                                                                                                                                                                                                |
| 16. | exp Relaxation Therapy/                                                                                                                                                                                         |
| 17. | exp Imagery, Psychotherapy/                                                                                                                                                                                     |
| 18. | exp Relaxation/ or exp Muscle Relaxation/                                                                                                                                                                       |
| 19. | "relaxation therapy".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] |
| 20. | meditation.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]           |
| 21. | "guided imagery".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]     |
| 22. | "verbal protocol".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]    |
| 23. | "music therapy".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]      |

|            |                                                                      |
|------------|----------------------------------------------------------------------|
| <b>24.</b> | 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 15                |
| <b>25.</b> | 11 or 12 or 13 or 14 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 |
| <b>26.</b> | 24 and 25                                                            |
| <b>27.</b> | exp music/                                                           |
| <b>28.</b> | 25 or 27                                                             |
| <b>29.</b> | 24 and 28                                                            |

#### CINAHL:

|             |                                                                                                                                   |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------|
| <b>S1.</b>  | (MH "Breast Feeding+")                                                                                                            |
| <b>S2.</b>  | (MH "Lactation") OR (MH "Lactation Disorders+")                                                                                   |
| <b>S3.</b>  | (MH "Milk, Human")                                                                                                                |
| <b>S4.</b>  | "human milk" OR breastmilk OR "breast milk" OR breastfeeding OR "breast feeding" OR breastfed OR lactation                        |
| <b>S5.</b>  | S1 OR S2 OR S3 OR S4                                                                                                              |
| <b>S6.</b>  | (MH "Relaxation") OR (MH "Relaxation Techniques+") OR (MH "Muscle Relaxation") OR (MH "Progressive Muscle Relaxation (Iowa NIC)") |
| <b>S7.</b>  | (MH "Guided Imagery")                                                                                                             |
| <b>S8.</b>  | (MH "Simple Relaxation Therapy (Iowa NIC)")                                                                                       |
| <b>S9.</b>  | (MH "Meditation") OR (MH "Meditation (Iowa NIC)")                                                                                 |
| <b>S10.</b> | (MH "Music Therapy") OR (MH "Music Therapy (Iowa NIC)")                                                                           |
| <b>S11.</b> | "relaxation therapy" OR "music therapy" OR meditation OR "guided imagery" OR "verbal protocol"                                    |
| <b>S12.</b> | (MH "Music")                                                                                                                      |
| <b>S13.</b> | S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12                                                                                         |
| <b>S14.</b> | S5 AND S13                                                                                                                        |

#### Web Of Science:

|            |                                                                                                                                    |
|------------|------------------------------------------------------------------------------------------------------------------------------------|
| <b>#1.</b> | ALL=("relaxation therapy" OR "relaxation technique" OR music OR imagery OR meditation OR "verbal protocol" OR "muscle relaxation") |
| <b>#2.</b> | ALL=("breast milk" OR breastmilk OR lactation OR "human milk" OR breastfeeding OR "breast feeding" OR breastfed)                   |
| <b>#3.</b> | #1 AND #2                                                                                                                          |

**Table A.2: Template data extraction form**

|                                                                              |
|------------------------------------------------------------------------------|
| Study Identification                                                         |
| Sponsorship source                                                           |
| Country                                                                      |
| Setting                                                                      |
| Comments                                                                     |
| Authors name                                                                 |
| Institution                                                                  |
| Email                                                                        |
| Address                                                                      |
| Conflicts of Interest                                                        |
| Trial registration details                                                   |
| Protocol published before end of recruitment?                                |
| Methods                                                                      |
| Design                                                                       |
| Group                                                                        |
| Power calculation comments                                                   |
| Randomisation method                                                         |
| Statistical analysis method, including methods for dealing with missing data |
| Deviation from protocol?                                                     |
| Population                                                                   |
| Inclusion criteria                                                           |
| Exclusion criteria                                                           |
| Group differences                                                            |
| Time period of recruitment                                                   |
| Number of participants randomised per arm                                    |

|                                                                          |
|--------------------------------------------------------------------------|
| Age (years, mean)                                                        |
| Socioeconomic status/education/family structure                          |
| Gestational age (week, mean)                                             |
| Birthweight (gram, mean)                                                 |
| Interventions                                                            |
| Nature of relaxation intervention (including provider/self-administered) |
| Theory of intervention effect                                            |
| Dose of intervention recommended/planned                                 |
| Length of intervention period recommended/planned                        |
| Any modifications to relaxation material, cultural congruency            |
| Single or multicomponent                                                 |
| Compliance                                                               |
| Outcomes                                                                 |
| Per arm: mean, SD, n                                                     |
| Per arm: median, IQR, n                                                  |
| Per arm: n/N                                                             |

**Table A.3: Detailed summary of each included study**

| Study                                                                                    | Setting                                                                            | Inclusion                                                                                   | Baseline                                                                                         | Intervention/control                                                                                                                                                                                                                      | Outcomes included                                                                                                                                                                                                                                                       |
|------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Ak et al 2015<sup>175</sup></b><br><br><b>Crossover RCT</b>                           | N = 30<br><br>University Hospital NICU<br><br>India<br><br>2012-2013               | Infants:<br>Born <34 weeks' PMA, admitted to NICU                                           | Mean age 24 years.<br><br>45% had caesarean birth                                                | Intervention: 30 mins Indian flute music (headphones, supervised)<br><br>Randomised to listening at morning or afternoon session each day<br><br>Control: same conditions, no music                                                       | Milk quantity: milk expressed during 15 min expressing session at 11am or 4pm, each day for four days<br><br>Salivary cortisol: Before and after the intervention expressing session on the fourth day                                                                  |
| <b>Chawanpaiboon et al 2021<sup>198</sup></b><br><br><b>Parallel group RCT</b>           | N = 620<br><br>University Hospital postnatal ward<br><br>Thailand<br><br>2018-2019 | Elective caesarean<br><br>Infant: Singleton, born at ≥37 weeks' PMA, not admitted to NICU   | Mean age 31-34 years, most had degree<br><br>Infants: Mean PMA 38 - 39 weeks'                    | Intervention: Eight mins calming Thai song (headphones).<br><br>Two intervention arms were a) listening solely in the delivery theatre, or b) also during bf for the next two days<br><br>Control: standard care                          | Exclusive bf: Telephone at 7 and 14 days; 1, 2, 3 and 6 months after birth<br><br>Note, the two intervention arms have been pooled meta-analysis, producing a 2:1 allocation ratio                                                                                      |
| <b>Dabas et al 2019<sup>200</sup></b><br><br><b>Parallel group RCT</b>                   | N = 57<br><br>University Hospital NICU<br><br>India<br><br>2017                    | 18-45 years<br><br>Infant: Singleton, born at 26 – 33 weeks' PMA, admitted to NICU          | Mean age 29-30 years, most had degree or above<br><br>Infants: 28-40% had BW <1kg                | Intervention: 30 mins relaxation practice including yoga breathing exercises and progressive muscle relaxation<br><br>Instruction: Guided practice for 30 minutes each day, for 10 consecutive days<br><br>Control: Standard care         | At day 10:<br><br>Parental Stress Scale for the NICU (PSS: NICU, score 1-5 on each of three dimensions)<br><br>Perinatal Anxiety Screening Scale (PASS. 31 items, score 0-93)<br><br>Milk quantity: milk expressed with electric pump until empty, divided by frequency |
| <b>Dagli &amp; Celik 2022<sup>165</sup></b><br><br><b>Non randomised crossover trial</b> | N = 73<br><br>University hospital NICU,<br><br>Turkey<br><br>Year not reported     | No hearing or vision loss, Turkish speaking<br><br>Infant: Born <34 weeks' PMA, ≥7 days old | Mean age 31.2 years<br><br>40% primip<br>41% employed<br>42% have degree,<br>92% caesarean birth | Intervention: 30 mins calming Turkish music (headphones, supervised)<br><br>Instruction: Around 1pm, day three of study<br><br>Control: Same conditions, no music, day one. Note a second intervention arm is not included in this review | Milk quantity: volume expressed in 15 mins with double electric pump (halfway through intervention)<br><br>Spielberger State Trait Anxiety Index (STAI, 20 items, score 0-80 for each sub). Not reported whether outcome is state or trait score                        |



| Study                                                                     | Setting                                                                              | Inclusion                                                                                                    | Baseline                                                                                                                                    | Intervention/control                                                                                                                                                                                                                                                                                                                                               | Outcomes included                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|---------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dib et al 2022</b> <sup>163,195</sup><br><br><b>Parallel group RCT</b> | N = 72<br><br>Urban capita<br><br>UK<br><br>2019-2021                                | Intention to bf for >6wk, non-smoker, English speaking<br><br>Infant: singletons born at 34 to 38 weeks' PMA | Mean age 33 years. 68% primip 73% degree or higher 55% White, 16% Black, 17% Asian 49% caesarean birth<br><br>Infants: mean PMA 36.5 weeks' | Intervention: lactation focused recording. Modified version of a commercial recording<br><br>Instruction: listen while breastfeeding, at least daily for 2 weeks<br><br>Control: standard care with breastfeeding support.<br><br>Neither group was aware that they were in a randomised trial or that other participants were receiving/not receiving relaxation. | Outcomes at two and 6wk after birth<br><br>Milk intake: test weighing infant before and after each feed<br><br>Infant diary: three-day diary record, recorded at fifteen min epochs<br><br>Infant weight: Intergrowth-21 growth charts<br><br>Perceived Stress Scale (PSS; 14 items, 0-40 score). Edinburgh Postnatal Depression Score (EPDS; 10 items, 0-30 score)<br><br>Salivary cortisol: midmorning. ELISA.<br><br>Breastmilk: express from full to empty at midmorning and ≥2 hours from a previous feed. Sample mixed. |
| <b>Feher et al 1989</b> <sup>202</sup><br><br><b>Parallel group RCT</b>   | N = 55<br><br>University and urban private NICUs<br><br>USA<br><br>Year not recorded | Three to five days after birth<br><br>Infant: Born preterm and expected to be in NICU for ≥10 days           | Mean age 25 to 27 years<br><br>43-68% primip<br><br>Infants: Mean PMA 31-32 weeks'                                                          | Intervention: 20 mins lactation focused recording<br><br>Instruction: listen once daily until expressing assessment<br><br>Control: standard care                                                                                                                                                                                                                  | Milk quantity: single expressing session of undefined length, one week after enrolment (mean day 8 of study)<br><br>Milk fat: sample expressed a minimum of two hours after previous expression, targeted at one week after enrolment                                                                                                                                                                                                                                                                                         |

| Study                                                                           | Setting                                                            | Inclusion                                                                                                       | Baseline                                                                                                                                   | Intervention/control                                                                                                                                                                                                                                                                                                                                      | Outcomes included                                                                                                                                                                                                                                                                                                                                                                                             |
|---------------------------------------------------------------------------------|--------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Keith et al 2012<sup>203</sup></b><br><br><b>Parallel group RCT</b>          | N = 162<br><br>NICU, Urban<br><br>USA<br><br>Year not recorded     | Non-smokers<br><br>Infant: Born <38 weeks' PMA or critically ill and admitted to NICU                           | Mean age 25 to 29 years<br><br>Twins 13-16%<br><br>Infants: Mean PMA 31-33 weeks'                                                          | Intervention 1: 12 mins lactation focused recording. Modified version of a commercial recording. Intervention 2: additional music behind the voice recording<br><br>Instruction: listen while expressing milk as often as possible, for 14 days. Given mp3 player<br><br>Control: standard care. One further intervention arm not included in this review | Milk quantity: expressing diary, participant record of milk volume from day 1 to 14 of study<br><br>Milk fat: 1ml sample of composite milk collected daily around noon from day 1 to 14 of study<br><br>Note, the two intervention arms included have been pooled for meta-analysis, producing a 2:1 allocation ratio                                                                                         |
| <b>Kittithanesuan et al 2017<sup>199</sup></b><br><br><b>Parallel group RCT</b> | N = 304<br><br>Provincial hospital<br><br>Thailand<br><br>2013     | Vaginal birth<br><br>Infant: Born at ≥37 weeks' PMA                                                             | Mean age 25-26 years<br><br>49% prior bf<br><br>Infants: Mean PMA 38.5 weeks'                                                              | Intervention: 11 mins calming Thai song<br><br>Instruction: played in the delivery room once<br><br>Control: Standard care                                                                                                                                                                                                                                | Colostrum score: nurses in delivery room performed a single compression of each breast within the first hour of life and after the first feed, before 2 hours of life. Scored 0-3 by yield                                                                                                                                                                                                                    |
| <b>Massa et al 2022<sup>164</sup></b><br><br><b>Parallel group RCT</b>          | N = 70<br><br>University Hospital NICU<br><br>USA<br><br>2018-2019 | Planned to provide milk for at least a month<br><br>Infant: Born at 24 to 32 weeks' PMA, less than 48 hours old | Mean age 27 to 29 years<br><br>52% Black 41-48% primip 16 – 21% multiple birth 58 – 66% caesarean birth<br><br>Infants: Mean PMA 30 weeks' | Intervention: free access to a commercial mindfulness-based meditation app for postpartum period<br><br>Instruction: Use daily for 20 mins, signposted to tracks related to lactation and postpartum recovery<br><br>Control: Standard care (received access to the app after study end)                                                                  | 24-hour milk yield, expressing frequency, expressing method: day 9 of the infant's life, participant diary<br><br>Day 10 to 16 after birth: Edinburgh Postnatal Depression Scale (EPDS); Spielberger State-Trait Anxiety Index (STAI); Perceived Stress Scale: NICU (PSS: NICU); Breastfeeding Self-Efficacy Scale short form for NICU (BSES-SF-NICU)<br><br>Any breastmilk: telephone at 28 days after birth |

| Study                                                                                        | Setting                                          | Inclusion                                                                                               | Baseline                                                                                                        | Intervention/control                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Outcomes included                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|----------------------------------------------------------------------------------------------|--------------------------------------------------|---------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Mohd Shukri 2019</b> <sup>172,196</sup><br><br><b>Parallel RCT</b>                        | N = 64<br><br>Urban Malaysia, 2014               | Primip, non-smoker, exclusive bf at 2wk after birth<br><br>Infant: Born at ≥37 weeks' PMA and BW ≥2.5kg | Most were 26-30 years old with a degree and a goal to bf for more than 19 months<br><br>75% had a vaginal birth | Intervention: lactation focused recording, translated into Malay with minor amendments (English and Malay versions available to participants). Modified from a commercial recording<br><br>Instruction: listen to the recording while bf or expressing at least daily from 2 to 14wk of age<br><br>Control: standard care, including bf education material from the researcher.<br><br>Neither group was aware that they were in a randomised trial or that other participants were receiving/not receiving relaxation. | At 2 and 6wk after birth unless specified:<br><br>Cohen's Perceived Stress Score (PSS), Edinburgh Postnatal Depression Scale (EPDS), Beck Anxiety Inventory (BAI; 21 items and score 0-63): two, six and 12-14wk after birth<br><br>Breastmilk intake: deuterium isotope to mother at two & 12wk<br><br>Breastmilk and salivary cortisol: before and after listening to intervention, morning<br><br>Infant diary: three-day diary, 5 min epochs<br><br>Infant weight: two, six and 12wk. World Health Organisation 2006 growth charts.<br>Body comp: deuterium (infant) 12wk |
| <b>O'Connor et al 1998</b> <sup>201</sup><br><br><b>Non-randomised intervention al study</b> | N = 38<br><br>Urban USA<br><br>year not reported | 20-40 years old<br><br>Infant: full term, healthy                                                       | Mean age 29 to 32 years<br><br>78-93% White<br><br>23-67% primip                                                | Intervention: individual relaxation session, taped for future use<br><br>Instruction: use tape for two weeks<br><br>Control 1: standard care; Control 2: 20 mins conversation about life (both received relaxation after study)                                                                                                                                                                                                                                                                                         | Symptom Checklist-90-R (SCL-90-R, 90 item including subscales for anxiety and depression) at baseline and 2 weeks<br><br>Note, the two control groups were pooled for synthesis                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Perez-Blasco et al 2013</b> <sup>169</sup><br><br><b>Parallel group RCT</b>               | N = 26<br><br>Urban Spain<br><br>2012            | Lactating                                                                                               | Mean age 34 years<br><br>57% primip<br><br>Infants: Mean age 10.8 months                                        | Intervention: two hour mindfulness training with babies. Each session included several 10 min meditations<br><br>Instruction: attend weekly, for eight weeks<br><br>Control: standard care (mindfulness instruction after study end)                                                                                                                                                                                                                                                                                    | Depression, Anxiety and Stress Scale (DASS 21; each of the three subscales is 7 item, score 0-21): self-report at baseline and 11 weeks into the study (3wk after the intervention was complete)                                                                                                                                                                                                                                                                                                                                                                              |

| Study                                                                       | Setting                                                                           | Inclusion                                                                                                                   | Baseline                                                                                                                     | Intervention/control                                                                                                                                                                                                                        | Outcomes included                                                                                                                                                                                                                                                     |
|-----------------------------------------------------------------------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Ramesh et al 2020</b> <sup>176</sup><br><br><b>Parallel group RCT</b>    | N = 81<br><br>University Hospital postnatal<br><br>India<br><br>Year not reported | Primip, 19-35 years old<br><br>Infant: Born at ≥37 weeks' PMA                                                               | Most were 21-25 years old and had high school education                                                                      | Intervention: 15 mins Indian classical flute music and encouraged to sing lullabies for 15 mins<br><br>Instruction: Listen/sing twice a day for 45 days<br><br>Control: Standard care                                                       | Timing of lactogenesis II (milk 'coming in'): UNICEF checklist each day for four days                                                                                                                                                                                 |
| <b>SefidHaji et al 2022</b> <sup>167</sup><br><br><b>Parallel group RCT</b> | N = 100<br><br>Regional hospital<br><br>Iran<br><br>2020                          | Primip, intention to bf, no use of galactagogues or drug abuse, no serious health issues<br><br>Infant: 34 to 36 weeks' PMA | Mean age 29 – 30 years.<br><br>64 – 91% moderate economic status<br><br>Weight 61 - 62kg<br><br>Infants: mean PMA 34.8 weeks | Intervention: 30 mins Iranian lullabies (headphones, supervised)<br><br>Instruction: Around noon, once daily for six days<br><br>Control: standard care with bf instruction. Note, a second intervention arm was not included in the review | Milk quantity: volume expressed in 15 mins with double electric pump on day one and six of study (for intervention group - half way through the intervention period)<br><br>Breastmilk constituents: 2ml sample taken from the milk expressed as above (mixed sample) |
| <b>Shabnam et al 2021</b> <sup>166</sup><br><br><b>Parallel group RCT</b>   | N = 70<br><br>University Hospital<br><br>Iran<br><br>2016-2017                    | Infant: Born with BW 2 to 2.5kg, without severe illness                                                                     | Infants: Mean PMA 36.5 – 37 weeks'<br><br>Mean BW 2.3kg                                                                      | Intervention: 5-7 mins classical music<br><br>Instruction: Listen during feeds three times per day for four weeks. Both mother and infant should be able to listen to the music                                                             | Time bf: mother record for 28 days, advised to use timer<br><br>No other description of how maternal reported outcomes defined. All recorded for 28 days<br><br>Weight, height and head circumference measured by professionals day 28                                |
| <b>Varisoglu et al 2020</b> <sup>174</sup><br><br><b>Parallel group RCT</b> | N = 44<br><br>University Hospital NICU<br><br>Turkey<br><br>2017-2018             | Primip<br><br>Infant: Born at 28 to 34 weeks' PMA and admitted to NICU                                                      | Mean age 28-29 years<br><br>Infants: Mean PMA 32 weeks'                                                                      | Intervention: 15 mins calming Turkish music<br><br>Instruction: Listen twice a day while expressing for three consecutive days<br><br>Control: standard care                                                                                | Milk quantity: volume expressed in 15 mins during each of two sessions (morning and afternoon).<br><br>Spielberger State Trait Anxiety Index (STAI): baseline and day four<br><br>Maternal salivary cortisol: baseline and day four                                   |

| Study                                                                    | Setting                                    | Inclusion                                                                                                                   | Baseline                                                                                                                                                                            | Intervention/control                                                                                                                                                                                                                                                                                                                                                    | Outcomes included                                                                                                                                                                                                                                                                                                                                                                                                                           |
|--------------------------------------------------------------------------|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Yu et al 2023</b> <sup>162,197</sup><br><br><b>Parallel group RCT</b> | N = 96<br><br>Urban China<br><br>2019-2020 | Primip, intention for exclusive bf for at least two months, non-smoker<br><br>Infant: singleton born at 34 to 37 weeks' PMA | Mean age 30 years<br><br>70% had degree or higher<br><br>42% were in the lowest income bracket (<200,000 CNY/ year)<br><br>37% caesarean birth<br><br>Infants: mean PMA 36.1 weeks' | Intervention: lactation focused recording. Modified from a commercial recording and translated into Mandarin<br><br>Instruction: listen while bf or expressing milk at least daily for 7 weeks<br><br>Control: standard care.<br><br>Neither group was aware that they were in a randomised trial or that other participants were receiving/not receiving relaxation.   | Outcomes at two and 6wk after birth:<br><br>24 hour milk intake: test weighing infant before and after each feed for 48 hours (+ volume of breastmilk bottles)<br><br>Infant diary: three-day diary record, recorded at 15 min epochs<br><br>Infant weight: Intergrowth-21 growth charts<br><br>Cohen's Perceived Stress Scale; Beck Anxiety Index (Chinese versions)<br><br>Breastmilk: express with a hand pump the first 20ml (foremilk) |
| <b>Yu et al 2019</b> <sup>108</sup><br><br><b>Crossover RCT</b>          | N = 20<br><br>Urban China<br><br>2018      | Primip, 23-45 years, currently bf, non-smoker<br><br>Infant: Less than two years old                                        | Mean age 32 years<br><br>Infants: Mean age 7.6 months                                                                                                                               | Intervention 1: lactation focused recording, shortened from commercial recording and translated into Mandarin<br><br>Intervention 2: music (new age or traditional)<br><br>Instruction: sit down without the infant for 10 mins, once only for each arm (separated by 1-3 days)<br><br>Control: no stimuli. Note several other intervention arms not included in review | Visual analogue scale (VAS): 10cm anchored as completely relaxed to completely unrelaxed. Participant report before and after the treatment. Measured in mm and scored as 0-10<br><br>Physiological measurements of systolic blood pressure, diastolic blood pressure, heart rate and fingertip temperature, measured before and immediately after the 10 min treatment.                                                                    |

RCT = randomised controlled trial. PMA = post-menstrual age. BW = birthweight. Mins = minutes. Wks = weeks. Bf = breastfeeding. Primip = primiparous

**Table A.4: Data for individual meta-analysis outcomes**

|                                              | Outcome                                                                                                                                        | N   | MD                    | 95% CI         | Risk of Bias  |
|----------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|-----|-----------------------|----------------|---------------|
| <b>Human milk quantity</b>                   |                                                                                                                                                |     |                       |                |               |
| <b>Ak et al 2015</b> <sup>175</sup>          | 15 minutes of expression on day 4 of study (day after birth not reported)                                                                      | 29  | 0.5 mL                | 0.2 to 0.8*    | High          |
| <b>Dabas et al 2019</b> <sup>200</sup>       | One expressing session on mean day 14 after birth (averaged from 24hr record)                                                                  | 50  | 15.1 mL               | 3.5 to 26.7    | Some concerns |
| <b>Dagli &amp; Celik 2022</b> <sup>165</sup> | 15 minutes of expression on day 3 of study (the “second or following weeks of lactation”)                                                      | 73  | 3.5 mL                | 1.5 to 5.5*    | High          |
| <b>Dib et al 2022</b> <sup>163</sup>         | 24 hour intake from the breast at 6-8 weeks after birth                                                                                        | 7   | 184 g                 | -162 to 530    | High          |
| <b>Feher et al 1989</b> <sup>202</sup>       | One expressing session (no specified length) on approximately day 11 after birth                                                               | 55  | 34.7 mL               | 6.1 to 63.3    | High          |
| <b>Keith et al 2012</b> <sup>203</sup>       | 24 hour expressed milk record on day 14 of study (approximately day 16 after birth)                                                            | 120 | 396.1 mL <sup>†</sup> | 276.9 to 515.2 | Some concerns |
| <b>Massa et al 2022</b> <sup>164</sup>       | 24 hour expressed milk record on day 9 after birth                                                                                             | 54  | 132.2 mL              | -99.3 to 363.7 | Some concerns |
| <b>Mohd Shukri et al 2019</b> <sup>172</sup> | 24 hour intake from the breast (averaged over two weeks) at 12-14 weeks after birth                                                            | 19  | 145.0 g               | -60.1 to 350.1 | Some concerns |
|                                              | 24 hour intake from the breast (averaged over two weeks) at 12-14 weeks after birth, adjusted for infant sex and intake at 2 weeks after birth | 19  | 226.5 g               | 24.0 to 430.0  | Some concerns |
| <b>Sefid Haji et al 2022</b> <sup>167</sup>  | 15 minutes of expression on day 6 after birth                                                                                                  | 66  | 4.9 mL                | 2.5 to 7.3     | Low           |
|                                              | Change between 15 minutes of expression on day 1 and 6 after birth                                                                             | 66  | 5.0 mL                | 4.2 to 5.7     | Low           |
| <b>Varisoglu et al 2020</b> <sup>174</sup>   | 15 minutes of expression on day 4 of study (day after birth not reported)                                                                      | 38  | 16.2 mL**             | -0.3 to 32.7   | High          |
|                                              | Change between 15 minutes of expression on day 3 and day 4 of study (day after birth not reported)                                             | 38  | 10.8 mL               | 2.5 to 19.1    | High          |
| <b>Yu et al 2023</b> <sup>162</sup>          | 24 hour intake from the breast (averaged over 48 hours) at 7-9 weeks after birth                                                               | 27  | 1.0 g                 | -28.5 to 30.5  | High          |
| <b>Human milk fat</b>                        |                                                                                                                                                |     |                       |                |               |
| <b>Dib et al 2022</b> <sup>163</sup>         | Change in composite milk fat from week 2-3 to week 6-8 after birth at around 11am (g/100 mL)                                                   | 47  | 0.35                  | -0.6 to 1.2    | Some concerns |
|                                              | Composite milk fat at week 6-8 after birth at around 11am (g/100 mL)                                                                           | 56  | -0.1                  | -0.8 to 0.5    | Some concerns |
| <b>Feher et al 1989</b> <sup>202</sup>       | Composite milk creatocrit on approximately day 11 after birth, unspecified circadian timing (%)                                                | 45  | 0.40                  | -1.0 to 1.8    | High          |
| <b>Keith et al 2012</b> <sup>203</sup>       | Composite milk fat on day 14 of study at around midday (approximately day 16 after birth; g/L)                                                 | 119 | 10.7 <sup>†</sup>     | -32.4 to 53.8  | Some concerns |
| <b>Sefid Haji et al 2022</b> <sup>167</sup>  | Change in composite milk triglyceride from day 1 to 6 after birth at around midday (mg/dL)                                                     | 66  | 32.9                  | 25.5 to 40.3   | Low           |
|                                              | Composite milk triglyceride on day 6 after birth at around midday (mg/dL)                                                                      | 66  | 28.8                  | -9.3 to 66.8   | Low           |
| <b>Yu et al 2023</b> <sup>162</sup>          | Change in foremilk fat from week 1 to 8 after birth at around 10am (g/100 mL)                                                                  | 92  | 0.12                  | -0.0 to 0.3    | Low           |
|                                              | Foremilk fat at week 8 after birth at around 10am (g/100 mL)                                                                                   | 92  | 0.12                  | -0.1 to 0.3    | Low           |

|                                                         | Outcome                                                                                                      | N  | MD      | 95% CI                    | Risk of Bias  |
|---------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|----|---------|---------------------------|---------------|
| <b>Infant weight, length and head circumference</b>     |                                                                                                              |    |         |                           |               |
| <b>Dib et al 2022</b> <sup>163</sup>                    | Change in weight SDS from 2 to 6-8 weeks after birth                                                         | 67 | 0.40    | 0.09 to 0.71              | Low           |
|                                                         | Change in length SDS from 2 to 6-8 weeks after birth                                                         | 55 | -0.2    | -0.65 to 0.25             | Some concerns |
| <b>Mohd Shukri et al 2019</b> <sup>172</sup>            | Change in weight SDS from 2 to 12 weeks after birth                                                          | 63 | 0.76    | 0.29 to 1.23              | Low           |
|                                                         | Change in length SDS from 2 to 12 weeks after birth                                                          | 63 | -0.09   | -0.52 to 0.34             | Low           |
| <b>Shabnam et al 2021</b> <sup>166</sup>                | Weight at 28 days (grams)                                                                                    | 58 | 492.4   | 303.2 to 681.6            | High          |
|                                                         | Length at 28 days (cm)                                                                                       | 58 | 4.89    | 3.41 to 6.37              | High          |
|                                                         | Head circumference at 28 days (cm)                                                                           | 58 | 0.27    | -0.53 to 1.07             | High          |
| <b>Yu et al 2023</b> <sup>162</sup>                     | Change in weight SDS from 1 to 8 weeks after birth                                                           | 96 | 0.51    | 0.15 to 0.87              | Low           |
|                                                         | Change in length SDS from 1 to 8 weeks after birth                                                           | 96 | 0.38    | -0.1 to 0.8               | Low           |
| <b>Infant time awake and alert (not crying/fussing)</b> |                                                                                                              |    |         |                           |               |
| <b>Mohd Shukri et al 2019</b> <sup>172</sup>            | Time (mins) per day (averaged from three day diary of 15 minute epochs) at 6-8 weeks after birth             | 37 | -56.0   | -131.0 to 19.0            | High          |
| <b>Shabnam et al 2021</b> <sup>166</sup>                | Time (hours per day) averaged over 28 days                                                                   | 58 | 0.8     | 0.5 to 1.1                | High          |
| <b>Yu et al 2023</b> <sup>162</sup>                     | Time (mins) per day (averaged from three day diary of 15 minute epochs) at 8 weeks after birth               | 51 | 19.4    | -48.5 to 87.2             | High          |
| <b>Infant time sleeping</b>                             |                                                                                                              |    |         |                           |               |
| <b>Dib et al 2022</b> <sup>163</sup>                    | Time (mins) per day (averaged from three day diary of 15 minute epochs) at 6-8 weeks after birth             | 33 | -4.4**  | -102.5 to 93.7            | High          |
| <b>Mohd Shukri et al 2019</b> <sup>172</sup>            | Time (mins) per day (averaged from three day diary of 15 minute epochs) at 6-8 weeks after birth             | 37 | 82.0    | 16.0 to 149.0             | High          |
| <b>Shabnam et al 2021</b> <sup>166</sup>                | Time (hours) “during 28 days” (unclear format or period of recording)                                        | 58 | 0.9     | 0.69 to 1.12              | High          |
| <b>Yu et al 2023</b> <sup>162</sup>                     | Time (mins) per day (averaged from three day diary of 15 minute epochs) at 8 weeks after birth               | 51 | 32.7    | -49.2 to 114.5            | High          |
| <b>Infant time crying/fussing</b>                       |                                                                                                              |    |         |                           |               |
| <b>Dib et al 2022</b> <sup>163</sup>                    | Time (mins) per day (averaged from three day diary of 15 minute epochs) at 6-8 weeks after birth Time (mins) | 31 | -22.6** | -75.1 to 29.9             | High          |
| <b>Mohd Shukri et al 2019</b> <sup>172</sup>            | Time (mins) per day (averaged from three day diary of 15 minute epochs) at 6-8 weeks after birth             | 37 | 21.0    | -27.0 to 69.0             | High          |
| <b>Yu et al 2023</b> <sup>162</sup>                     | Time (mins) per day (averaged from three day diary) at 8 weeks after birth                                   | 41 | -14.0   | -53.2 to 24.3             | High          |
| <b>Anxiety (lactating mother/parent)</b>                |                                                                                                              |    |         |                           |               |
| <b>Dabas et al 2019</b> <sup>200</sup>                  | Perinatal Anxiety Screening Scale on mean day 14 after birth                                                 | 50 | -8.4    | -13.7 to -3.1             | Some concerns |
| <b>Dagli &amp; Celik 2022</b> <sup>165</sup>            | Spielberger State Trait Anxiety Index on day 3 of study (the “second or following weeks of lactation”)       | 73 | -5.3    | -8.5 to -2.1 <sup>‡</sup> | High          |

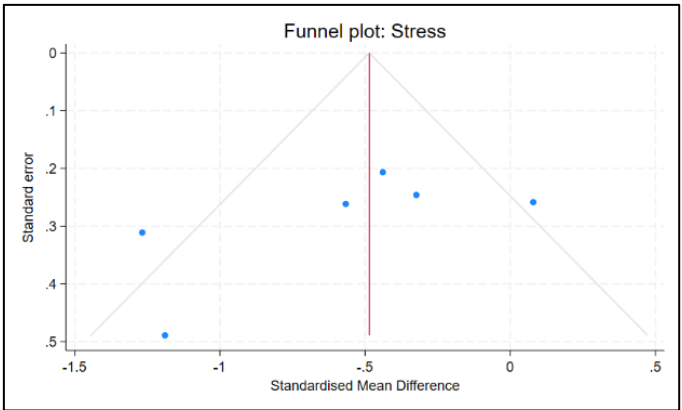
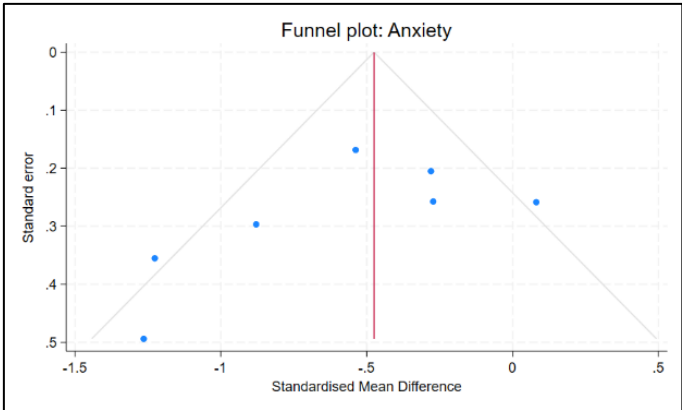
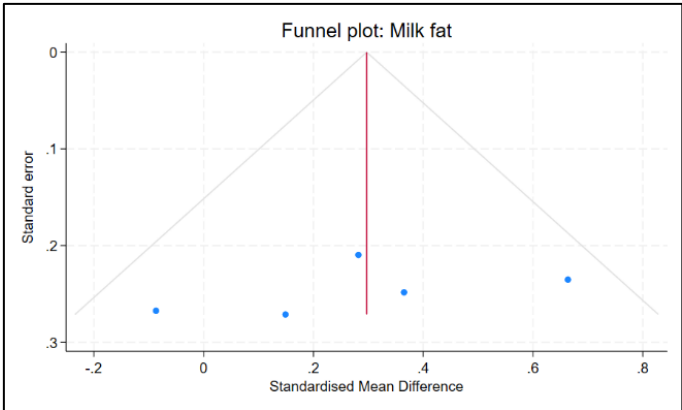
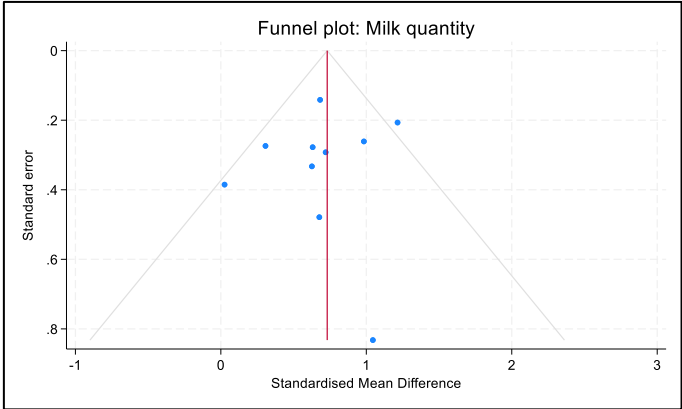
|                                               | Outcome                                                                                    | N  | MD           | 95% CI                           | Risk of Bias  |
|-----------------------------------------------|--------------------------------------------------------------------------------------------|----|--------------|----------------------------------|---------------|
| <b>Massa et al 2022</b> <sup>164</sup>        | Spielberger State Trait Anxiety Index on day 10-16 after birth                             | 60 | 1.8          | -9.8 to 13.4                     | Some concerns |
| <b>Mohd Shukri et al 2019</b> <sup>172</sup>  | Beck Anxiety Index at 12-14 weeks after birth                                              | 61 | -3.0         | -8.6 to 2.6                      | Some concerns |
| <b>O'Connor et al 1998</b> <sup>201</sup>     | Symptom Checklist-90-R anxiety subscale at 4-6 weeks after birth                           | 38 | -0.1         | Not reported but not significant | High          |
| <b>Perez-Blasco et al 2013</b> <sup>169</sup> | Depression, Anxiety and Stress (anxiety subscale) at mean 13 months after birth            | 21 | -4.8         | -8.3 to -1.2                     | Some concerns |
| <b>Varisoglu et al 2020</b> <sup>174</sup>    | Spielberger State Trait Anxiety Index on day 4 of the study (day after birth not reported) | 38 | -6.1         | -9.3 to -2.9                     | High          |
| <b>Yu et al 2023</b> <sup>162</sup>           | Beck Anxiety Index at 8 weeks after birth                                                  | 96 | -1.4         | -3.4 to 0.6                      | Some concerns |
|                                               | Change in Beck Anxiety Index between week 1 and week 8 after birth                         | 96 | -1.3         | -3.0 to 0.4                      | Some concerns |
| <b>Stress (lactating mother/parent)</b>       |                                                                                            |    |              |                                  |               |
| <b>Dabas et al 2019</b> <sup>200</sup>        | Perceived Stress Scale: NICU on mean day 14 after birth                                    | 50 | -0.7         | -1.0 to -0.4                     | Some concerns |
| <b>Dib et al 2022</b> <sup>163</sup>          | Perceived Stress Scale at 6-8 weeks after birth                                            | 67 | -1.7         | -4.3 to 0.9                      | Some concerns |
|                                               | Change in Perceived Stress Scale from 2 to 6-8 weeks after birth                           | 67 | -0.2         | -2.8 to 2.4                      | Some concerns |
| <b>Massa et al 2022</b> <sup>164</sup>        | Perceived Stress Scale: NICU on day 10-16 after birth                                      | 60 | 1.9          | -10.3 to 14.1                    | Some concerns |
| <b>Mohd Shukri et al 2019</b> <sup>172</sup>  | Perceived Stress Scale at 12-14 weeks after birth                                          | 61 | -3.1         | -5.9 to -0.3                     | Some concerns |
| <b>Perez-Blasco et al 2013</b> <sup>169</sup> | Depression, Anxiety and Stress (stress subscale) at mean 13 months after birth             | 21 | -8.5         | -15.1 to -1.8                    | Some concerns |
| <b>Yu et al 2023</b> <sup>162</sup>           | Perceived Stress Scale at 8 weeks after birth                                              | 96 | -2.8         | -5.3 to -0.2                     | Some concerns |
|                                               | Change in Perceived Stress Scale from 1 to 8 weeks after birth                             | 96 | -2.7         | -4.5 to -0.8                     | Some concerns |
| <b>Depression (lactating mother/parent)</b>   |                                                                                            |    |              |                                  |               |
| <b>Dib et al 2022</b> <sup>163</sup>          | Edinburgh Postnatal Depression Scale at 6-8 weeks after birth                              | 61 | -0.3         | -2.4 to 1.8                      | Some concerns |
|                                               | Change in Edinburgh Postnatal Depression Scale from 2 to 6-8 weeks after birth             | 54 | 0.5          | -1.3 to 2.3                      | Some concerns |
| <b>Massa et al 2022</b> <sup>164</sup>        | Edinburgh Postnatal Depression Scale on day 10-16 after birth                              | 60 | Med Diff: -1 | -5 to 2.5                        | Some concerns |
| <b>Mohd Shukri et al 2019</b> <sup>172</sup>  | Edinburgh Postnatal Depression Scale at 12-14 weeks after birth                            | 61 | -1.3         | -3.3 to 0.7                      | Some concerns |
| <b>O'Connor et al 1998</b> <sup>201</sup>     | Symptom Checklist-90-R depression subscale at 4-6 weeks after birth                        | 38 | 0.1          | Not reported but not significant | High          |
| <b>Perez-Blasco et al 2013</b> <sup>169</sup> | Depression, Anxiety and Stress (depression subscale) at mean 13 months after birth         | 21 | -1.2         | -4.3 to 1.9                      | Some concerns |



|                                                    | Outcome                                                                          | N  | MD    | 95% CI                     | Risk of Bias  |
|----------------------------------------------------|----------------------------------------------------------------------------------|----|-------|----------------------------|---------------|
| <b>Salivary cortisol (lactating parent/mother)</b> |                                                                                  |    |       |                            |               |
| <b>Ak et al 2015</b> <sup>175</sup>                | Units: nmol/L.<br>Collected on day 4 of the study (day after birth not reported) | 29 | 0.08  | -0.44 to 0.59 <sup>¥</sup> | High          |
| <b>Dib et al 2022</b> <sup>163</sup>               | Units: microgram/dL.<br>Collected at 6-8 weeks                                   | 49 | 0.02  | -0.02 to 0.06              | High          |
|                                                    | Units: microgram/dL.<br>Change from 2 to 6-8 weeks                               | 41 | -0.08 | -0.15 to -0.01             | High          |
| <b>Mohd Shukri et al 2019</b> <sup>172</sup>       | Units: microgram/dL.<br>Collected at 6 weeks after birth                         | 60 | -0.27 | -0.78 to 0.24              | Some concerns |
| <b>Varisoglu et al 2020</b> <sup>174</sup>         | Units: nmol/l.<br>Collected on day 4 of the study (day after birth not reported) | 38 | -0.44 | -1.08 to 0.21              | High          |

MD = mean difference. CI = Confidence Interval. Med Diff = median difference. Composite milk is a mixture of foremilk and hindmilk. \*Confidence interval calculated by review authors using paired test p value reported in publication (crossover trial) †pooled intervention groups (identified B and D in original report) \*\*provided by authors on request ¥Assuming unpaired data – conservative approach

**Figure A.1: Funnel plots for individual meta-analyses with more than four studies included**



## Appendix B: Documents supporting the parent involvement process (related to Chapter 4)

### Document B.1: Published report on parent involvement

Levene et al.  
International Breastfeeding Journal (2022) 17:69  
<https://doi.org/10.1186/s13006-022-00509-1>

International Breastfeeding  
Journal

#### RESEARCH

#### Open Access



# A report on parent involvement in planning a randomised controlled trial in neonatology and lactation – insights for current and future research

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#### Abstract

**Background:** Patient and Public Involvement (PPI) is a rich and valuable part of the process of planning, designing, carrying out and disseminating research. It is important to communicate PPI findings in detail so that the contributions of those involved are fully utilised and disseminated. The extended and iterative PPI process used within a neonatal randomised controlled trial related to the expression of breastmilk after very preterm birth is reported here.

**Methods:** Seven iterative stages of PPI were used. Stage 1 was informal PPI using historical interaction with parents and publicly available resources. Stage 2 was an online questionnaire open to parents of premature babies and advertised via a charity collaborator. Stage 3 was partnership with a charity collaborator. Stage 4 was a set of online panels focusing on study design and documents. Stage 5 was an interactive exercise to modify the trial intervention. Stage 6 is the presence of PPI contributors on the trial steering committee. Stage 7 is a dissemination panel. At each stage attention was paid to the diversity of participants involved, with strategies to increase the involvement of parents from under-reached groups.

**Results:** Six hundred and seventy-five participants responded at Stage 2, six parents were involved at Stage 4 and 12 parents at Stage 5. PPI contributed to the choice of study question, outcomes and produced a set of questions for future research. PPI impacted on the study design, with specific emphasis on reducing participant distress related to lactation, and reducing the burden of being involved in research at a time of significant stress.

**Conclusions:** PPI had a far-reaching influence on this neonatal randomised controlled trial during the planning and design phase, which reinforces the importance of PPI at the earliest stages of the research cycle. The online questionnaire format elicited an unexpectedly deep and broad pool of transferable insights, which will have an impact on future research focus and design in the area of lactation and prematurity. Approaches to increasing PPI involvement from under-reached populations are important and can be successful despite resource constraints.

**Keywords:** Public engagement, Breastfeeding, Prematurity, Human milk

#### Background

##### What is PPI?

Patient and Public Involvement (PPI) is the process of involving those who benefit from research in planning, performing and disseminating research. In a neonatal context, the term patient covers the parents and family

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members of a newborn as well as older children or adults who experienced neonatal conditions [1]. PPI at every stage of research is becoming more established [2, 3], but brings with it a complex power dynamic and a need for specific resource allocation at any early stage, often before funding applications are written [4]. Early stage researchers, particularly students, may therefore encounter challenges to integrating effective PPI into research plans [5].

Positive impacts of PPI include increasing trial recruitment or retention [2], refining the research question to ensure it has maximum utility [6], correcting bias and incorrect assumptions [7], and pre-emptively identifying potential problems [8]. In addition, PPI can be viewed through a rights-based lens as a social good [6] and through a political lens as necessary for the accountability and legitimacy [9] of science. It is important to pay attention to potential discrimination in whose voices are heard, which will involve particular emphasis on involving marginalised groups [6, 10, 11].

### **Trial context**

This paper reports the PPI process associated with designing a neonatal randomised controlled trial (RCT) in the field of breastmilk feeding after a very preterm birth (less than 32 weeks of completed gestation). Complications arising from preterm birth are the leading cause of neonatal death globally [12]. Babies born very preterm have higher mortality rates through infancy and beyond [13], as well as increased rates of disability [14]. Increasing the amount of mother's own milk (MOM) given to very preterm babies is an important intervention to lessen morbidity and mortality, particularly in relation to necrotising enterocolitis, a serious gut condition [15]. However very preterm babies cannot directly breastfeed due to their immaturity, so mothers must establish and then maintain their breastmilk supply by mechanical expression, which is challenging and anxiety provoking [16, 17]. Mothers of very preterm babies have a high risk of low milk supply [18–22]. The RCT will test a relaxation intervention for mothers and assess its impact on breastmilk feeding and mental health outcomes. The term breastmilk feeding refers to provision of breastmilk to the infant by any route (for example gastric tube, cup, bottle or direct breastfeeding).

### **Reporting PPI**

When significant resources have been devoted to PPI and new insights gained, it is important that the PPI process is reported in detail [23] so that the contributions of those involved are fully utilised and disseminated to other researchers, avoiding 'invisible' or 'black box' PPI that wastes the time and efforts of PPI

collaborators [24]. PPI reports also stimulate researchers to challenge their own practice, which can be tokenistic [4, 25], and can share lessons learned, which are common and transferable [4]. PPI reports should cover short term outcomes (what was said and learnt), medium term outcomes (changes resulting from this learning) and long term impacts, for example on the future research agenda, as these can all be used by the wider research community [7]. Such reports are becoming more frequent [23, 26, 27].

This report aims to examine the short, medium and long-term impacts of an extended PPI process in the design of a neonatal RCT, providing useful insights for the design and structure of future research.

### **Overview of PPI stages**

There were five major PPI stages at the planning and design stage of the RCT (stage one to five), one stage supports the ongoing trial management (stage six) and one further stage is planned for dissemination (stage seven). These stages are described in Table 1 and their relationship with the trial lifecycle is shown in Fig. 1. This paper will focus on stages one to five as these are the stages that are often missing from PPI implementation [4] (See Table 1).

Figure 1 shows that early PPI can influence the entire research cycle, and that later PPI stages can deepen and refine elements that have been raised in earlier, broader stages. It also illustrates the practical problem that early PPI occurs before trial funding is obtained, and must therefore be performed with minimal resource or using alternative funding strategies [4] (See Fig. 1).

### **Integrated methods and results: structure and influences of PPI stages 1 to 5**

#### **Stage 1 – Informal PPI**

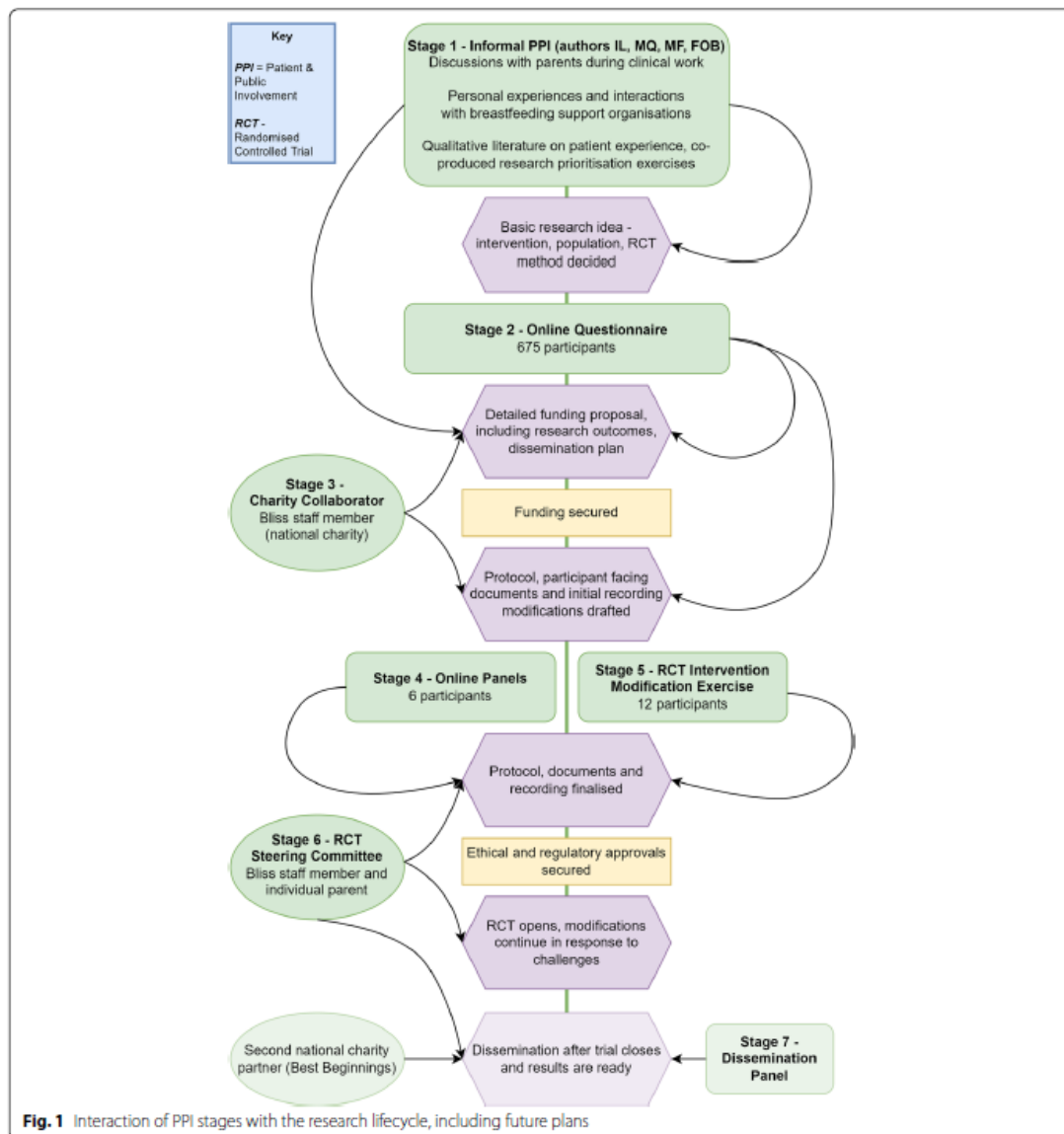
##### **Structure**

The primary researcher (IL) is a neonatal clinician who has listened to parents of preterm babies during clinical work over ten years of practice, with a particular interest in lactation. IL also has personal experience of expressing breastmilk for prolonged periods and extensive involvement in breastfeeding support organisations and peer support networks as a service user and volunteer.

Qualitative literature and published PPI were used as another way to hear the voice of parents of very preterm infants [16, 17, 26, 28–32] and co-produced research prioritisation exercises [33] were consulted. All of these factors inform an understanding of parental experiences, priorities and values that can be used as a springboard for formal PPI planning.

**Table 1** Description of PPI stages complete, ongoing and planned

| Stage                                                | Description                                                                                                                                                                                                                              | Demographic characteristics                                                                                                                       |
|------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| 1. Informal PPI (complete)                           | Informal discussions with families over ten years of neonatal practice; personal experiences with expressing breastmilk and breastfeeding support communities; published qualitative literature and partnership prioritisation exercises | Varied family characteristics. Four of the authors involved at this stage had experiences related to expressing milk.                             |
| 2. Online Questionnaire (complete)                   | Online questionnaire with free text questions and prioritisation exercises. Advertised via Bliss (national charity for sick and preterm infants)                                                                                         | 675 contributors. 71% experienced birth before 32 weeks of pregnancy. 95% white ethnicity                                                         |
| 3. Charity Collaborator (complete)                   | Bliss staff members involved in creation of funding application and RCT protocol                                                                                                                                                         | Two staff members                                                                                                                                 |
| 4. Online Panels (complete)                          | Two evening online panels. Contributors sent documents to review in advance. Discussion of trial design and documents encouraged. Role description provided in advance and payment offered as per INVOLVE guidance [27]                  | Six contributors. Two from Black and other minority ethnic groups. One was under 25 years old                                                     |
| 5. RCT Intervention Modification Exercise (complete) | Contributors sent two draft intervention recordings. Focused questionnaires with feedback and recommendations. Role description provided in advance and payment offered as per INVOLVE guidance                                          | 12 contributors. Four from Black and other minority ethnic groups. Two contributors with English as a second language. One was under 25 years old |
| 6. RCT Steering Committee (ongoing)                  | PPI contributors on the Trial Steering Committee attend meetings and are consulted when difficulties occur. Role description provided in advance and payment offered as per INVOLVE guidance.                                            | One parent and one Bliss staff member                                                                                                             |
| 7. Dissemination Panel (planned)                     | A PPI dissemination panel will be formed to discuss strategy and create resources. Best Beginnings (a national charity) will co-create printed resources for neonatal units                                                              | Study participants who consented for further contact will be invited                                                                              |



### Influence

This informal PPI foundation influenced decisions about the intervention area (relaxation recording), the population (mothers of very preterm babies), the method (RCT) and the broad outcome set (relating to expressed milk volume and breastfeeding experiences).

### Stage 2 – online questionnaire

#### Structure

An online questionnaire was advertised through social media channels of Bliss, a large charity supporting families with sick and preterm babies in the United Kingdom. Bliss provides parent and professional facing resources,



individual support, national advocacy and supports research. The questionnaire was designed to elicit opinions from parents of preterm infants with experience of lactation on research priorities related to lactation, what outcomes are most important, potential practical and ethical difficulties of a trial and where parents obtain lactation information. All contributors were sent an executive summary of the findings of the questionnaire as feedback. No specific target number of respondents had been pre-specified however the questionnaire was closed after 1 week due to the overwhelming response.

Six hundred and seventy-five people responded to the questionnaire (respondents were not asked their gender but all had experience of lactation). Forty-seven percent had an infant born between 28 and 32 weeks of gestation ("very preterm"). A quarter (24%) had an infant born at less than 28 weeks' gestation ("extremely preterm"). Half (51%) had provided breastmilk to their infant for 6 months or less. A quarter (23%) had provided breastmilk for more than 1 year. 95% of respondents reported white ethnicity. The sample included 30 mothers from an ethnic minority background and 52 who were under 25 years old at the time of the preterm birth.

#### **Influence – choice of outcomes**

The first key influence on the research design was in the choice of outcomes. When asked what questions they had about how to optimally express for and breastfeed a preterm baby, the most common question was how to express more milk and one in five respondents raised this in their free text response (21%). When asked about lactation problems, 27% reported that they had low milk supply and/or could not express as much milk as they wanted. This focus on milk volumes was seen in all groups, including younger mothers and those from ethnic minorities.

Respondents were asked to rank seven pre-defined research outcomes identified through the Stage 1 PPI process. Three outcomes were dominant; being able to express more milk (top priority for 32%), being able to breastfeed for longer (top priority for 25%) and feeling that milk supply was more secure (top priority for 19%).

These results gave a strong direction that the primary outcome of the study should be expressed milk yield, and that duration of breastmilk feeding was an important secondary outcome.

Although milk yield was dominant when asked about problems experienced and questions they would like answered, it was not a dominant response when asked what success or improvement meant in the area of lactation, there was very little consensus on this issue and no single type of response was given by more than 10% of respondents. Table 2 shows the diversity of responses (See Table 2).

The stress experienced in relation to lactation was evident from the language used by respondents. Frequently used descriptive words were "struggle" (used 87 times), "problem" (81 times), "difficult" (72 times), "stress" (60 times), "hard" (50 times) and "pressure" (24 times). Descriptions of stress and impact on mental health often revolved around milk volumes and milk supply.

*"Constantly anxious about expressing enough milk"*

*"[pumping] added to my post-natal trauma and depression. I will never forget how much of a failure. .. I felt."*

*"I was heartbroken that I couldn't do the one thing I thought I should have been able to do!"*

**Table 2** Examples of the diverse range of answers to questions about what 'success' means to parents in relation to breastmilk feeding in prematurity

| Answers relating to the nature of the breastmilk feeding experience       | Answers related to the duration of breastmilk feeding                                                                                    |
|---------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Being able to express more milk                                           | For longer than actually experienced                                                                                                     |
| Minimising the effect of expressing on time spent with the baby           | Until complementary feeds ("solids") were introduced                                                                                     |
| Providing exclusive maternal milk                                         | Until discharge from hospital                                                                                                            |
| Any direct breastfeeding (getting the baby to latch to the breast at all) | Until the baby was perceived as less vulnerable to infection (for example after winter or when the baby had reached a particular weight) |
| Exclusive direct breastfeeding                                            | Six months from birth                                                                                                                    |
| The mother being more happy or relaxed in relation to lactation           | A year from birth                                                                                                                        |
| Feeling well supported                                                    | Until the baby was a toddler                                                                                                             |
| Improving the baby's weight gain                                          | Until the baby or child decided they wanted to stop breastfeeding                                                                        |
| Feeling listened to and trusted by staff                                  |                                                                                                                                          |

This wide variety of issues raised, including mental health, expressing efficiency and duration of breastfeeding, informed the choice of secondary outcomes. As anxiety was the overwhelming emotion expressed in relation to expressing milk it was a clear choice for psychometric assessment. It was clearly important to many families to breastmilk feed beyond discharge, whereas discharge is often used as a surrogate marker of breastmilk feeding success due to the relative ease of measurement. However, there was no agreement on a single definition of successful breastfeeding for preterm babies; decision making around duration encompassed not only limitations experienced (such as milk supply or growth pattern), but also parental perceptions of the baby's medical status and fragility, previous plans and the conflicts experienced between lactation plans and mental health. Therefore, the research design incorporated established public health goals, any breastmilk and exclusive breastmilk, at time points that were logistically or physiologically relevant. The first timepoint chosen was 36 weeks' post-menstrual age (4 weeks before the estimated date of delivery), as a standardised timepoint commonly used in neonatal research. The second timepoint chosen was 4 months' corrected age (4 months after the estimated date of delivery), as a time point representing the likely maximum milk supply required and an age where complementary feeds are indicated in almost all cases for very preterm babies [34]. It was also decided to ask participants for their breastmilk feeding goals at baseline to inform subgroup analysis.

#### ***Influence - addressing anxiety over milk yield in study design***

A third of respondents (29%) reported at least one concern about the idea of a research study looking at expressing and breastfeeding in preterm babies. The predominant concern was that many mothers have feelings of guilt, judgement, inadequacy and anxiety related to lactation and that being in a study where volumes of expressed milk are monitored and questions asked about breastfeeding and mental health outcomes could exacerbate this, increasing a feeling of pressure or trauma.

Respondents made suggestions to mitigate these risks, which included:

- Communicate sensitively, respectfully and tactfully
- Ensure that families do not feel pressured over breastmilk outcomes but give gentle encouragement, reassurance, support and empowerment
- Be explicit that many mothers have difficulty expressing and breastfeeding and this is 'ok'
- Minimise face to face questions as this could impose more pressure and guilt than filling in details electronically

- Ensure mothers feel heard

These responses were used to draft the participant facing documents, using appropriate language to reduce any feeling of pressure and ensure that participants were aware that some people can find it hard to express milk. They also supported the decision to predominantly use a more impersonal electronic interface rather than face-to-face data collection.

#### ***Influence - minimising burden for participants***

Some respondents reported a concern that the research study would be an extra burden to participants, taking time away from their baby and intruding into their lives at a stressful time. They recommended making the process as easy as possible to minimise this burden. Expressing milk was clearly already a considerable burden that was in direct competition with maternal needs, including factors that might be protective of their mental health such as sleep and time with family, so adding an extra component of yield measurement was a concern.

*"... often a choice between sleeping and expressing which leads to horrendous guilt when choosing the former and risking health to prioritise the latter"*

*"Expressing was just difficult due to the time spent between hospital/home/school run/eating, sleeping and showering!"*

In response to these concerns, milk yield monitoring was minimised to three or four time points (depending on gestation at birth), rather than a daily log as used in some studies. Study design was built around electronic data entry, with participants receiving a personalised website link to enter data, in an attempt to make the process as quick and easy as possible. Text message and email reminders were automated for additional efficiency.

A tension exists between the desire for high accuracy assessment of milk yield, using weight, and the burden on participants, where reading the volume of milk from the container would impose less burden than using a weighing scale. A decision was made to prioritise the use of weights for accuracy while also minimising the number of timepoints that participants are asked to do this. However, it remained clear through the trial that weighing milk was difficult for some participants and for future trials, volume reporting may be preferable where medical grade containers with appropriate volume markings are available. It may also be preferable for research staff to measure milk yield rather than participants, although this could interfere with milk provision for the baby unless trained staff are available at all times.



An area of tension between scientific priorities and the burden on parents at a time of heightened stress was the timing of recruitment. PPI contributors raised concerns that recruiting in the first days after preterm birth would cause distress. However, there are strong physiological reasons to suspect that interventions to improve milk supply need to occur as early as possible after birth [35–37]. Many neonatal trials recruit participants in the first days after birth [38] and some in the first hours after birth where this is required due to a need for urgent action [39]. A decision was made to allow recruitment up to day four after birth to balance these factors.

#### **Influence – additional data analysis**

Contributors had many questions about milk expression, summarised in Table 3 in order of frequency raised. More detail is available in supplementary Table 1 (see Additional file 1). These questions influenced the choice of outcomes for a planned secondary analysis of the data gathered in the RCT, and is also a source of influence for planning future research (See Table 3).

#### **Influence - dissemination plans**

The questionnaire asked for details about where parents found information about expressing for and breastfeeding their preterm baby. Multiple sources of information were reported, both formal and informal (Fig. 2). In response to predefined options, the majority (87%) of respondents said that they received information from neonatal unit staff and 40% used printed information found in the neonatal unit. 36% received information from maternity staff, such as a midwife, and 19% used social media for this purpose (See Fig. 2).

Free text responses listed a wide variety of social media channels and websites used for information (more than 50). Respondents used Instagram, Twitter, Facebook, Pinterest, YouTube and apps as well as websites as sources of information.

An initial dissemination plan was made using these findings. This focused predominantly on dissemination to clinical staff, particularly neonatal nurses and neonatal infant feeding leads as the key professionals transmitting information to families. A partnership with national charity Best Beginnings is planned to include the trial results, along with wider information about optimal expressing, on printed cards that can be attached to breast pumps in neonatal units. Best Beginnings is a charity providing accessible resources to parents, including parents of sick and preterm infants. A short video is planned to disseminate findings directly to the public, which will utilise the list of social media settings provided by PPI contributors.

#### **Influence - future research topics**

The distinct area of how to successfully transition from expressing (with gavage feeding, where milk is put directly into the stomach via a tube) to direct breastfeeding was frequently raised. Specific questions for future research are listed in Table 4, in order of frequency raised by respondents. More detail is available in supplementary Table 2 (See Additional file 2 and Table 4).

#### **Stage 3 – charity collaborator**

##### **Structure**

UK national charity Bliss has supported the research project from an early stage. One staff member collaborated on the creation of the funding application, trial protocol and trial documents and supported dissemination of the online questionnaire to parents. A second staff member

**Table 3** Questions for future research on breastmilk expression, from PPI contributors, in order of frequency mentioned

|                                                                                                                                                                                                          |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| How to increase or maintain milk yield ( $n=87$ ; including food, drink, medication, herbal remedies ( $n=17$ ), methods focusing on connection to the baby ( $n=8$ ) and mechanical methods ( $n=23$ )) |
| How frequently to express ( $n=50$ )                                                                                                                                                                     |
| How long to express for at each session/optimal pattern of expressing ( $n=30$ )                                                                                                                         |
| How best to relax/feel comfortable or happy while expressing milk ( $n=25$ )                                                                                                                             |
| How much milk should be targeted/expected ( $n=23$ )                                                                                                                                                     |
| How best to store breastmilk ( $n=11$ )                                                                                                                                                                  |
| The impact of delayed first expression/best timing ( $n=10$ )                                                                                                                                            |
| How to minimise and manage over-supply ( $n=9$ )                                                                                                                                                         |
| Personalised likelihood of being able to express good volumes of milk ( $n=9$ )                                                                                                                          |
| The need for and impact of sterilisation processes for pump equipment ( $n=7$ )                                                                                                                          |
| How to reduce the occurrence of blocked milk ducts and mastitis ( $n=4$ )                                                                                                                                |
| Personalised expectations for the time for milk to 'come in' (lactogenesis II) ( $n=4$ )                                                                                                                 |
| The impact of antenatal expressing ( $n=3$ )                                                                                                                                                             |

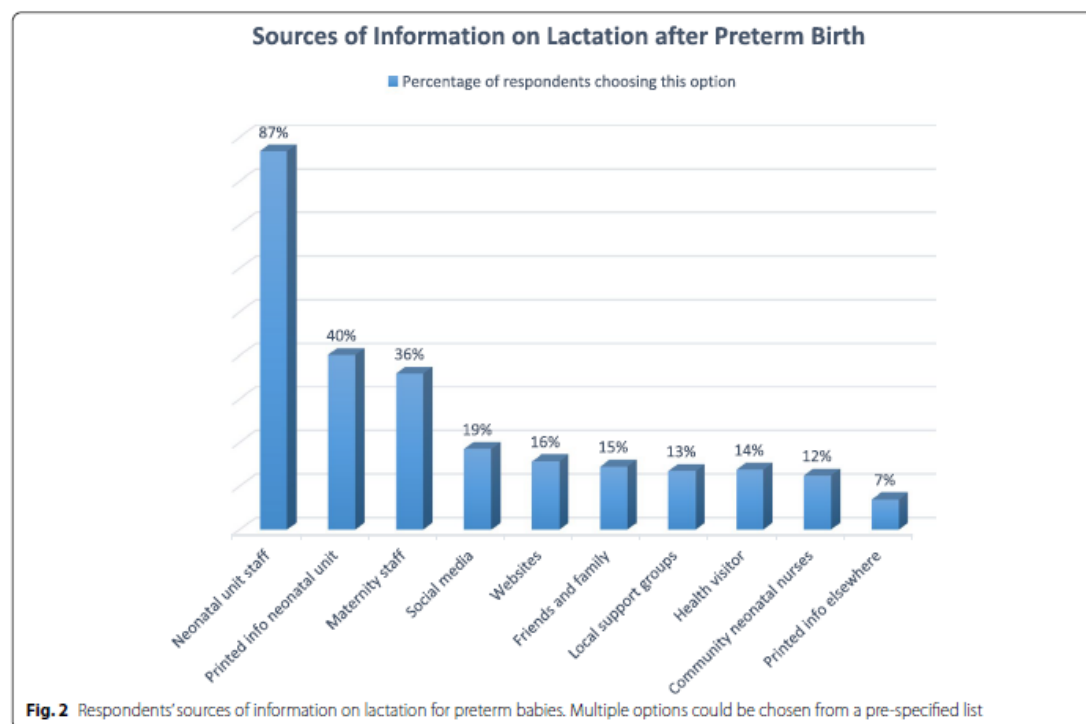


**Table 4** Questions for future research on direct breastfeeding in prematurity, from PPI contributors; in order of frequency raised

|                                                                                                                                                             |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| How to help preterm babies attach to the breast ( <i>n</i> = 50)                                                                                            |
| How to make decisions on the need for gavage supplements when transition to direct breastfeeding has started ( <i>n</i> = 19)                               |
| When to try direct breastfeeding for the first time ( <i>n</i> = 19)                                                                                        |
| How to transition the breasts from expressing to direct breastfeeding ( <i>n</i> = 19)                                                                      |
| The effect of "technology" (for example nipple shields, bottles, pacifiers) on the transition to direct breastfeeding ( <i>n</i> = 17)                      |
| How best to support mothers and partners' lactation, physical and emotional needs ( <i>n</i> = 12)                                                          |
| Personalised likelihood of success at transitioning to any/exclusive direct breastfeeding, and how long the transition is expected to take ( <i>n</i> = 10) |
| What normal behaviour is for a breastfed preterm baby – for example feeding frequency, night waking, crying ( <i>n</i> = 8)                                 |
| Impact of tongue-tie in prematurity ( <i>n</i> = 1)                                                                                                         |

sits on the Trial Steering Committee. Bliss receives funding for their time, as recommended by an increasing number of research funders.

#### **Influence**

Input centred on prioritising the mental health and physical needs of parents throughout recruitment and participation in the study, for example, defining an appropriate response to high scores on mental health questionnaires. Bliss also facilitated the very high response rate to the online questionnaire described in Stage 2, through social media dissemination.

#### **Stage 4 – online panels**

##### **Structure**

A subset of 49 questionnaire respondents who had given consent for further contact and whose infants were under 32 weeks' gestation at birth were invited in November 2020 to help review trial documents. This included all respondents who were under 25 years old at the birth of their baby or from ethnic minority groups. A formal role description was provided with the offer of associated payment in line with INVOLVE guidance [40].

Two panels were set up using videoconferencing software. Both were scheduled in an evening slot timed with the hope that contributors' children might be asleep. Panellists were reassured that they could come and go according to their children's needs. They were asked in advance whether they had any special requirements to maximise participation and sent documents to read in preparation.

Eleven mothers wanted to be involved in the online panels (22% of those invited) and were sent trial documents. Four contributors attended one of two online panels and two further mothers sent written feedback instead (12% of those invited). Two were from Black and other minority ethnic groups and one was under 25 years old. There was a range of gestational age experiences and one mother had experienced neonatal bereavement.

Initial activities were targeted at putting participants at ease, sharing their background and setting ground rules. Each document was discussed in turn and then pre-specified targeted questions were asked on particular terminology and issues that the researcher had experienced while writing the trial protocol. Finally, there was an opportunity for contributors to comment on the trial design and raise other points. Panellists were offered the opportunity to see final trial documents integrating their suggestions after the process was complete, as feedback.

It was also planned to discuss the documents in person one to one with parents currently in a neonatal unit to focus on demographics that were under-represented in the online questionnaire - for example mothers from non-European countries, teenage mothers and mothers from lower socioeconomic backgrounds. Unfortunately, this was not possible because of the SARS-CoV-2 pandemic restricting non-clinical presence in the hospital.

#### ***Influence – language and focus***

Panellists' feedback led to changes in language used and new documents being created, for example, a short introductory leaflet that would be less overwhelming than the full Participant Information Sheet. Free-text questions were added to the participant entered Case Report Forms to make them feel more accessible and encourage a relationship where the trial participants would feel seen as individuals. Resources were added to the end of each Case Report Form directing people to sources of support related to lactation, mental health and having a preterm baby.

#### ***Influence – choice of psychometric instrument***

Panelists were shown a range of psychometric instruments covering both depression and post-traumatic stress (PTS) reactions. The anxiety instrument had already been chosen (the Spielberger State Trait Anxiety Index) and because a limited number of secondary

outcomes could be included within the constraints of the sample size, one further instrument needed to be chosen. There was a clear preference for assessing PTS reactions and for the Post-Traumatic Check List for DSM-5 due to the tone of the language used in the questions. This preference for PTS assessment over depression was because contributors felt that PTS reactions are common after very preterm birth, poorly identified and are a priority for research.

#### ***Influence – additional training needs***

One panellist focused on the need for specific bereavement training for research staff because of the risk of participants experiencing the loss of their baby, or one of their babies for mothers of multiples. All research staff were recommended to undertake bereavement training and some reported the ways in which they used this in talking to bereaved parents within the trial.

#### ***Stage 5 – RCT intervention modification exercise***

##### ***Structure***

All respondents who had given consent for further contact were invited in January 2021 to help choose and modify the relaxation recording to be used as the RCT intervention (approximately 250 invitations). The invitation emphasised that people from minority groups were particularly welcomed. A formal role description was provided with an offer of associated payment in line with INVOLVE guidance [40].

Sixty-two people responded to an invitation to be involved in the intervention modification and choice exercise (25% of those invited). Twelve were selected to include a range of gestational ages at birth and to include people from diverse backgrounds and numbers were limited due to PPI payment costs budgeted. Maternal age ranged from 25 to 48 years. One third of the group came from a minority ethnic background and two spoke English as a second language.

Two draft audio files were sent to contributors, both had been used in previous RCTs [41–43]. Contributors were asked for detailed feedback, both open ended and specific and to choose their preferred recording. Once modifications were complete, all contributors were sent feedback on the impact of their involvement.

##### ***Influence – intervention choice and modification***

One recording was clearly preferred by the majority of contributors. The recording script was modified based on three key areas identified by PPI contributors, reducing a feeling of pressure and judgement on having a low milk supply; language and imagery appropriate for sick, immature babies; and language and imagery appropriate for women with traumatic pregnancy and birth experiences.

An example of contributor feedback (emphasis added):



*“The mention of uterus stood out as a trigger word, even now so many years after I had my daughter. I don’t think I would want to be thinking specifically about my uterus as that was linked to trauma. ... There was also a mention of expressing being the number 1 priority, this felt a bit pressured in that moment.”*

### Summary of influence and impact

Key impacts of PPI for this trial were in defining the study outcomes, minimising participant burden and anxiety through trial design, modifying the intervention and informing a dissemination plan. The key impact for future work is providing research questions identified by parents.

Table 5 describes the nature of the influence of PPI on the research project using the Public and Patient Engagement Evaluation Tool (PPEET [44]) for inspiration. The GRIPP2 reporting checklist for PPI reports [45] is also provided as supplementary material (see Additional file 3 and Table 5).

### Discussion

#### Strengths and weaknesses

Patient and Public Involvement had a far-reaching influence on the study during the planning and design phase,

which reinforces the importance of PPI at the earliest stages of the research cycle [4]. The online questionnaire format elicited an unexpectedly deep and broad pool of insights to influence trial design and the number of people involved was much larger than other published PPI reports [5, 26, 27]. However a known weakness of using social media to recruit participants [46] is that it is less effective at including marginalised voices, for example, families from ethnic minorities and communities with lower socioeconomic status. This is a frequent theme in PPI, where the people who are easiest to engage are often the most privileged [10, 11]. Thus 95% of online questionnaire respondents were white, whereas only 71% of people giving birth in the UK are white [47].

However, the very large response to the online questionnaire meant that even though harder to reach groups were under-represented proportionally, their voices are still present, the involvement of more than 50 young mothers and 30 mothers from ethnic minorities is a very positive achievement within a PPI setting. Asking about contributor demographics in the online questionnaire meant that minority voices could be preferentially studied and invited for further steps of PPI work. This meant that a third of the panellists and recording exercise contributors were from ethnic minority backgrounds and several were under 25 years old. Despite the strength of

**Table 5** Areas where PPI influenced the research. Inspired by the Public and Patient Engagement Evaluation Tool (PPEET)

| Type of influence                                           | Amount of influence | PPI stage involved                                                                                           | Comment                                                                                                                                  |
|-------------------------------------------------------------|---------------------|--------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Choice of population and intervention category              | Moderate            | Informal PPI (stage 1)                                                                                       | Set before formal PPI started                                                                                                            |
| Choice of trial outcome measures                            | Large               | Informal PPI (stage 1)<br>Online Questionnaire (stage 2)<br>Charity Collaborator (stage 3)                   | Formal PPI informed the choice of specific outcomes from a range of possibilities and their definitions (for example timepoint assessed) |
| Areas for exploratory analysis                              | Moderate            | Informal PPI (stage 1)<br>Online Questionnaire (stage 2)                                                     | Formal PPI confirmed areas of focus and provided more detail on questions of interest                                                    |
| Trial design – minimising adding to anxiety over milk yield | Large               | Online Questionnaire (stage 2)<br>Charity Collaborator (stage 3)<br>Online Panels (stage 4)                  | Amendments to processes and documents                                                                                                    |
| Trial design – minimising burden for participants           | Large               | Online Questionnaire (stage 2)<br>Charity Collaborator (stage 3)<br>Online Panels (stage 4)                  | Amendments to processes and documents. Some areas of tension decided by researchers                                                      |
| Trial modifications in response to challenges               | Small               | Trial Steering Committee (stage 6)                                                                           | Consultation. Areas of disagreement decided by researchers                                                                               |
| Intervention content                                        | Very Large          | Informal PPI (stage 1)<br>Online Questionnaire (stage 2)<br>RCT Intervention Modification Exercise (stage 5) | Iterative changes with detailed PPI input. Some disagreement among participants – final decision by researchers                          |
| Dissemination plan                                          | Very Large          | Online Questionnaire (stage 2)                                                                               | Detailed list of vehicles of communication for dissemination provided by PPI, along with assessment of most significant channels         |
| Future research topics                                      | Large               | Informal PPI (stage 1)<br>Online Questionnaire (stage 2)                                                     | Broad range of questions posed                                                                                                           |

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this over-sampling approach, diversity of participants was restricted by the data gathered at the questionnaire stage, for example, respondents were not asked about income or education level, or protected characteristics such as disability and sexual orientation, so there are likely areas of under-representation that were not identified or over-sampled. In addition, intersectional communities are less likely to be represented, for example, people from ethnic minority groups who also have low incomes.

There were many aspects of best practice within the PPI process. For example, contributors were adequately compensated (according to INVOLVE guidance [40]) to reduce barriers to involvement and to show the value of their 'symbolic capital' [48] as experienced parents with a deep understanding of the trial context. Role descriptions were provided to create a shared understanding of expectations and live interaction was planned to maximise inclusion of this particular population with young children. However around two thirds of those who wanted to participate in online panels did not end up attending which shows the difficulty of providing accessible arrangements for parents of young children, and a range of available times or individual consultations may have been preferable.

A limitation of the PPI design for this project was that the scope and areas of influence of the partnership were predominantly directed and designed by the researcher. There was an unequal balance of power with PPI contributors unable to ensure that their concerns were acted upon in any areas of tension or disagreement. Therefore the level of participation was predominantly 'consultation' with some elements of 'partnership' rather than higher levels of 'delegated power' or 'citizen control' [49], these models would require a more fundamental investment in PPI integration into clinical trials units and academic departments.

#### Putting findings into context

Some actions resulting from Patient and Public Involvement in this trial are similar to the conclusions from other neonatal and paediatric PPI conducted while planning RCTs [26, 27]. For example, providing participant information in short formats to cater for parents with different information needs, a focus on compassionate language when describing low volumes of maternal milk [26] and giving participants the opportunity to describe their experiences more freely, rather than being constrained to validated questionnaires and numerical responses [27]. However the broad, foundational questions asked in the online questionnaire, and the very large number of responses at an early stage, meant that this PPI process has resulted in a deeper and wider set of transferrable insights than most reports [5, 26, 27]. Thus, in addition to practical changes to trial design, a wide range of future

research topics that parents want answers to has been presented, which can be a springboard for the research community.

Much further work needs to be done to answer parents' questions about how to express milk most effectively and efficiently for their very preterm babies while protecting their mental health, and how to best transition to direct breastfeeding, fulfilling their personal priorities and goals. Future trials seeking these answers can also implement the insights described here to choose meaningful outcomes, minimise parental anxiety and burden and design effective dissemination plans.

#### What could be done differently

The PPI process relied heavily on digital literacy and access because of the limitations imposed by the SARS-CoV-2 pandemic. Although this allowed a broader reach and in some cases may have increased accessibility for parents of young children, additional strategies are needed to include those in digital poverty and with communication barriers [10].

Future PPI should be structured to increase the power of PPI participants in the research process. Even without structural changes to integrate shared decision making, contributors can be given more opportunities to challenge the power dynamic by providing anonymous feedback on how the PPI was conducted [23], for example using the PPEET questionnaires [44].

Any weaknesses of this process were partially the result of Patient and Public Involvement taking place without initial funded resource, and partly due to the inexperience of the researcher, both are acknowledged barriers to high quality PPI within doctoral research [5], although seen at all levels [4]. University Departmental PPI networks and support systems could help overcome both resource pinch points and provision of expertise.

#### Conclusions

Investing time, expertise and financial resource into Patient and Public Involvement at an early stage can have a very significant impact on research, including randomised controlled trials. This may be particularly important in a high stress environment such as after very preterm birth and where the condition under study (lactation) is influenced by complex emotional and physical contributory factors. This report also demonstrates that under-representation of rarely reached groups is not inevitable within Patient and Public Involvement.

Much can be learnt from reporting Patient and Public Involvement processes and findings in full. This report may help other researchers plan effective and iterative PPI for their own research. Lessons learned can be

translated to future research in relation to the questions and outcomes that are important to parents in this field, as well as effective dissemination pathways. In addition, this detailed report emphasises to the academic community as well as to PPI contributors themselves, the high value and importance of their involvement.

#### Abbreviations

DSM: Diagnostic and Statistical Manual of Mental Disorders; GRIPP2: Guidance for Reporting Involvement of Patients and the Public (second revision); MOM: mothers' own milk; PPEET: Public and Patient Engagement Evaluation Tool; PPI: Public and Patient Involvement; PTS: Post traumatic stress; RCT: Randomised controlled trial; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2.

#### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13006-022-00509-1>.

**Additional file 1: Supplementary Table 1.** Detailed list of questions for future research on breastmilk expression. A table listing detailed questions for future research on breastmilk expression, submitted by 675 respondents to an online questionnaire for parents of premature babies [50].

**Additional file 2: Supplementary Table 2.** Detailed list of questions for future research on direct breastfeeding for preterm infants. A table listing detailed questions for future research on direct breastfeeding, submitted by 675 respondents to an online questionnaire for parents of premature babies.

**Additional file 3.** GRIPP2 short form reporting checklist. A completed GRIPP2 checklist (Guidance for Reporting Involvement of Patients and the Public).

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#### Authors' contributions

IL is the Chief Investigator of the RCT; she conceived the PPI process, led the activities and drafted the manuscript. BM is one of the Bliss staff members who provided PPI input as described in the paper, and had involvement in drafting the manuscript. FOB, MF and MQ are IL's doctoral supervisors; they participated in planning and overseeing the PPI process and have had input into the manuscript. FA provided input into the interpretation of results and drafting of the manuscript. All authors read and approved the final manuscript.

#### Authors' information

BM is employed by Bliss, a national charity for sick and premature infants. IL is chair of the Hospital Infant Feeding Network, a knowledge-sharing organisation for hospital professionals interested in infant feeding (voluntary position). IL is a trustee of a community charity, Oxfordshire Breastfeeding Support (voluntary position).

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#### Availability of data and materials

Not applicable.

#### Declarations

##### Ethics approval and consent to participate

The activities described here are defined as public patient involvement with research, rather than primary research. The Research Governance, Ethics and Assurance team in the University of Oxford confirmed that the activities did not need ethical approval or a formal consent process and can be reported and published within this context. The PPI activities informed the design of a clinical trial approved by the Bloomsbury Research Ethics Committee 21/LQ/0279.

##### Consent for publication

Not applicable – as outlined in the ethics and consent section above.

##### Competing interests

MF receives an unrestricted donation for research on infant nutrition from Philips (not related to this work). The remaining authors declare that they have no competing interests.

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## ***Document B.2: Modifications of the intervention recording***

### **Reasons for modification:**

- A. Switching from audience who are direct breastfeeding to audience who are exclusively expressing, separated from their baby for at least some of the day, have a high likelihood of low milk production and are sensitive to perception of pressure or judgement on the volume they can express/time spent expressing.
- B. Switching from audience of mothers of healthy babies to audience of mothers of immature babies with potentially life-threatening illness and high frequency of multiple birth.
- C. Switching from audience of mothers who have given birth at term and had relatively normal pregnancies to mothers with potentially traumatic pregnancy and birth (and therefore particularly sensitive to terms like 'natural' or concepts such as trusting one's body, and may feel disconnected from the baby.
- D. Improving cultural congruence, for example reducing 'American' tone, reducing the number of repetitions of the word 'relax' and incorporating specific suggestions from parents.

### **Modifications demonstrated with tracked changes (underlined/red text was added and struck through text was removed, in comparison to the original script supplied by Sheri Menelli):**

First, give yourself a moment to settle in to your surroundings and relax. Make sure you are as comfortable as you can be with the settings on the pump or your hand position. Now take a breath in.  
And hold it. And let it out with a sigh. Take in another breath. And hold it. And again, let it out with a sigh.

And once more take in a breath and hold it. And then let it out at your own pace. Just close your eyes and allow your eyelids to relax to the point where your muscles feel as though they could fade and disappear. Imagine that you are in your favourite place in nature. This is a safe and tranquil place. You can imagine the breeze gently caressing your body, the sound of a rushing stream. And you take a nice, big breath and smell clean air. The sun is a perfect temperature as it shines down upon you.



Feel how wonderful it is to just ~~relax and~~ let go. The muscles around your scalp relax. And that relaxation is moving down through your forehead. Just ~~soften~~~~relaxing~~ those muscles around the eyes and the eyelids. And allowing your eyelids to feel heavier.

It feels so nice to release all tension in your body. The relaxation continues. The relaxation continues to drift over your cheeks and through your nose. The mouth and the jaws release and let go and you notice your teeth parting slightly.

As the shoulders drop and the arms ~~loosen~~~~limp at the elbow~~, you notice your body is feeling more and more relaxed. ~~Softer and softer~~~~More and more relaxed~~. Even your mind is becoming more serene. Feel how nice it is to let the relaxation move into your heart and your chest and then down through your internal organs.

Let it move down through your ~~tummy~~~~uterus~~ and into your hips. You'll find that with every breath you exhale, you relax even more. The relaxation is floating all the way down through your legs and into your feet and your toes. And when you feel the relaxation in your toes you can even imagine that any tension, stress and fear that remains is leaving your body out through the toes and moving into the earth.

As you ~~have already realized~~~~know~~, ~~giving~~ breast~~milk~~~~feeding to~~ your baby is ~~an~~~~one of the most~~ important jobs ~~that you have~~, ~~even when it is just a drop~~. ~~This is the time you are setting aside for your baby~~~~It is important for both the health and wellbeing of you and your baby that you focus your attention only on your baby.~~

Cherish the time ~~to step away from the outside world~~~~that you are spending with your baby~~ and you will be well rewarded with more milk ~~and better health~~. Your body and mind need an opportunity to relax. This is your opportunity ~~to !~~. ~~Let~~~~Breastfeeding~~ ~~your milk flow is your number one priority~~. Your mind works so hard for you, so it's time to let your mind relax too. To allow you to let go of all of the other intrusive thoughts just imagine that you are in your favourite place in nature.

And in this place in nature you feel, see or sense a path. As you walk along this path it meanders until you find a large book in front of you. Open this book and now place all of your to-do lists, thoughts and concerns into this book. Free your mind of every thought. Except for the thought of cherishing ~~this time~~

~~with~~ your baby. Now close this book. You can go back and pick up the items in the book at another time, after you have finished ~~feeding your baby~~here.

You are one with nature. ~~Just as other mammals in the wild are able to provide enough milk for their babies, so too are you able to provide the perfect amount of milk for your baby.~~ Your body ~~can~~was ~~designed to~~ feed your baby. Even when there have been challenges, you can grow to trust your body and the milk it can make. ~~It grows your baby perfectly in your womb with all the miracles that that entails. You can trust yourself and your baby to breastfeed together in harmony.~~ You and your baby are together, here inside your mind in perfect harmony. A wave of relaxation floats through every cell of your body as you concentrate on thoughts of being in ~~and your baby have~~ skin to skin contact with your baby. That tiny body resting on your chest ~~contact~~ brings a calm and serenity to your mind and body. The thought of the baby's soft skin touching yours instantly causes the breasts to let down their milk.

And as you exhale and say to yourself 'my milk lets down', you feel the warmth and the tingly-ness of the milk flowing down into both breasts. Yes, the ~~perfect amount of~~ milk for your baby flows into your breasts. It brings you so much joy to know that each drop is a gift ~~you can provide that nutrition~~ for your baby. Imagine ~~your baby nuzzling~~ opens his or her mouth wide enough to latch on perfectly at your breast and smelling your milky smell. ~~As the baby makes that sucking motion~~ Picture ~~you holding~~ your baby comfortably in your arms, close to you. You and your baby together ~~are working in perfect harmony.~~ You are both so comfortable and so relaxed. The thought of the baby ~~making that sucking motion~~ so close brings more milk into your breasts.

You can grow to love your body. It does such a fantastic job of bringing ~~an abundance of~~ milk for ~~to~~ your baby. Milk flows ~~so easily~~ down the milk ducts, drop by drop, then more and more, little jets of milk joining together into a greater flow. There is no one right way to make milk – this is your way. You grow more ~~feel~~ confident every time you see-feed your baby. Providing your milk ~~Breastfeeding~~ is very fulfilling. It empowers you. You grow more ~~are so~~ comfortable with your body. ~~You find that whenever and wherever you need to breastfeed your baby, you feel confident and self-assured. You find that you are attracted to a variety of nutritious foods. It feels so good to eat healthy and eating healthy gives you so much more energy. You find yourself drinking plenty of water. Your body knows how to replenish itself.~~

~~The water you drink keeps your skin smooth and supple. You take such good care of your baby and yourself. And you remember to nourish yourself daily too. You deserve good health.~~

Expressing your milk~~Breastfeeding can be~~ easier and more comfortable with each passing weekday.

And you also find that you and your baby love the time that you spend together ~~while you are feeding~~.

Your bond ~~just~~ grows stronger. ~~You are so pleased with your healthy and strong body.~~ Your nipples, milk ducts and breasts are all strong and healthy. Your breasts can do a wonderful job of feeding your baby.

Now talk to your baby. Remind your baby that ~~he or she is~~they are wanted, loved and welcomed. Let the baby know ~~that you love being a mom~~what it means to be their mother ~~and that you are so happy to~~and provide milk for ~~them~~him or her. You are so new to this and you need to give yourself~~Your little one needs plenty of patience. He or she is so new to this world. Your baby needs extra patience as she or he~~you learns how to ~~breastfeed~~become their mother.

As your milk flows~~breastfeed~~, you choose happy thoughts that make you feel good. You'll find that these happy thoughts allow you to feel centred. Filling you with peace and joy. You are so perfectly balanced. You enjoy breastfeeding~~providing milk for your baby~~. ~~You and your baby are so happy together.~~ Take time now to thank your body. Thank your body for each drop of~~the~~ milk it produces for your baby. Thank your body for your good health. Thank your mind for clearing so that you can dedicate this precious time to your baby.

If you prefer to stay here relaxing for a while, then that's ok. If you are expressing one breast at a time you might want to switch to the other breast now. Just count yourself up from one to five when you feel ready to finish this meditation~~do so~~. If you feel ready to finish this meditation now, I'm going to count up from one to five and when I reach five you will be wide awake. And it's now time to come back to full conscious awareness. And we'll do this slowly. Just allowing your eyes to remain closed until we reach the count of five. At one, coming up, up, up. Good, at two let your fingers and toes begin to move. At three, feeling more and more energy as you continue to come up, taking a big stretch and a deep breath. And at four let your eyes clear beneath your lids, ~~let the whites of your eyes get whiter now~~. And at five open your eyes, feeling plenty of energy, feeling great, feeling wonderful, having lots of milk coming into your breasts now.

## Appendix C: Published randomised trial protocol (related to Chapter 5)

Levene et al. *Trials* (2022) 23:611  
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Trials

### STUDY PROTOCOL

### Open Access



# Comparing the effect of a lactation-specific relaxation and visualisation intervention versus standard care on lactation and mental health outcomes in mothers of very premature infants (the EXPRESS trial): study protocol for a multi-centre, unmasked, randomised, parallel-group trial

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#### Abstract

**Background:** Premature birth is the leading cause of neonatal death and can cause major morbidity. Maximising the amount of maternal breastmilk given to very premature infants is important to improve outcomes, but this can be challenging for parents. Parents of infants receiving neonatal care also have high rates of anxiety and distress. There is growing evidence for the impact of maternal relaxation interventions on lactation, as well as mental health. The trial will assess whether a brief self-directed relaxation and visualisation intervention, recommended for use several times a day during expression of milk, improves lactation and mental health outcomes for mothers of very premature infants.

**Methods:** Multi-centre, randomised, controlled, unmasked, parallel-group trial with planned 132 participants who have experienced premature birth between 23 weeks and 31 weeks and 6 days of gestation and plan to express milk for at least 14 days. The primary outcome is the highest 24-h expressed milk yield recorded on any of day 4, day 14 or day 21 after birth. Secondary outcomes include exclusive breastmilk feeding at 36 weeks post-menstrual age and at 4 months after the estimated date of delivery, Spielberger State Trait Anxiety Index at day 21 and Post-traumatic stress Check List (for DSM 5) at day 21.

**Discussion:** Breastmilk feeding for premature infants is an important research priority, but there are few randomised controlled trials assessing interventions to help parents reach lactation goals in this challenging context. This trial will assess whether a no cost, easily scalable relaxation tool has a role in this setting. Given the lack of harm and potential for immediate dissemination, even a small benefit could have an important global impact.

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**Trial registration:** ISRCTN16356650. Date assigned: 19/04/2021.

**Keywords:** Newborn, Preterm, Breastfeeding, Breast milk, Maternal stress, Meditation, Relaxation therapy

## Background

There are an estimated 2.4 million infants born at less than 32 weeks gestation globally each year [1], and the rate of premature birth is increasing [2]. Complications arising from premature birth are the leading cause of neonatal death in the UK [3] and globally [2]. Premature infants also have increased risk of long-term disability, which increases as the gestational age at birth decreases [4]. Premature birth has a significant effect on parental mental health—systematic review estimates that 42% of parents have significant anxiety and 40% have significant post-traumatic stress reactions in the first month after birth [5]. Between 29 and 40% of mothers have depression in this timeframe [6]—all of these figures are higher than found after term, healthy birth [5, 6].

Infants born at less than 32 weeks gestation cannot fully orally feed from birth and are therefore given nutrition intravenously and/or directly into the stomach with an enteral tube. To provide breastmilk for their infants, mothers must express milk from the breasts for a prolonged period. Maximising the volume of maternal breastmilk given to these infants is associated with improved mortality, morbidity and long-term neurodevelopmental outcome [7–10]. Increasing the amount of breastmilk that mothers of very premature infants can express in the weeks after birth also leads to longer duration of breastmilk feeding [11, 12], with consequent well-known public health benefits for both infant and mother [13]. Breastmilk feeding is the provision of breastmilk by any route (for example, directly at the breast, by gastric tube or by bottle).

Despite motivation to provide expressed breastmilk for their infants, there is a high risk of poor milk supply, leading to non-exclusive breastmilk feeding [14–18] and an increasing failure to meet mothers' own goals for breastmilk provision as time goes on [19]. It is challenging to establish and then maintain a full milk supply through exclusive expressing [20, 21], which is exacerbated when pregnancy has been abbreviated and the breast tissue and hormonal milieu are therefore not at an optimal stage. Perinatal interventions associated with premature birth may also pose lactation challenges—such as provision of antenatal steroids, which also delay milk 'coming in' [22] (lactogenesis II), and caesarean birth.

Although breastmilk feeding in prematurity has been identified as a top ten research priority by the James Lind Alliance priority setting partnership [23], there are few randomised controlled trials (RCTs) related to

breastmilk expression [24]. A Cochrane review noted that one promising technique to improve expressed milk yield is guided relaxation and visualisation by the mother and recommended further research in this area [24]. Several neonatal unit RCTs [25–28] have shown increased expressed milk yield after a relaxation intervention, some with a dramatic effect (doubling the average yield) [25]. Studies in mothers of healthy babies have also shown reduced time to lactogenesis II [29], markers of improved milk transfer (such as infant weight gain) and reduction in maternal stress and anxiety [30, 31].

The theoretical basis of the effect of relaxation and visualisation on lactation is threefold—directly through the interaction of stress and lactation hormones, directly through the central nervous system and indirectly through behavioural factors. The major hormones of milk production and release are prolactin and oxytocin, which are closely connected to the wider web of hormones influencing perinatal mood [32]. For example, salivary cortisol is associated with prolactin level in mothers of very premature babies over 6 weeks of expressing milk [33] and measures of anxiety such as the State-Trait Anxiety Index (STAI) correlate with oxytocin level at 8 weeks after birth [34]. Relaxation interventions can reduce salivary and breastmilk cortisol [30, 35]. Thus it is possible that relaxation affects hormones such as cortisol, which then affect prolactin and oxytocin level.

Mental visualisation can trigger the milk ejection reflex (MER—the muscular ejection of milk), as seen in mothers with high spinal cord injury who can trigger MER with visualisation routines in the absence of any intact sensory pathways from the breast [36]. Mothers of premature infants often report difficulties with MER due to the reduced physical contact with their babies. Inhibition of MER leads to incomplete drainage of the breast and therefore secondary inhibitory feedback of milk production [37]. Targeting MER through visualisation therefore has the potential to increase milk yield.

Lastly, there may be behavioural mediators, whereby mothers who have less perception and manifestation of stress are more likely to interact positively with their infant [38] and have increased self-efficacy [39]. They may therefore put in place a more effective regime of frequent expressing and perform other actions with a positive influence on lactation such as spending more time in skin-to-skin contact with their infant.

A key weakness of existing studies is the focus on short-term outcomes, which in several cases have minimal



clinical significance [26, 27, 35, 40]. There are also significant concerns over risk of bias in many of the studies reported, including inadequate allocation concealment [26, 27], insufficient washout period for a crossover study [35] and selective reporting [27, 29, 41].

## Methods

### Aim and design

This is a multi-centre, unmasked, randomised controlled, parallel-group trial designed to determine if a self-directed relaxation and visualisation intervention for mothers of infants born between 23 weeks of gestation and 31 weeks and 6 days of gestation improves lactation and mental health outcomes. Both groups will receive standard clinical support for lactation provided by their neonatal unit staff. The intervention is a 12-min-long modified audio recording that participants are requested to listen to frequently while expressing breastmilk. The primary objective is outlined in Table 1.

Data is submitted directly by participants into case report forms and through participant responses to short message service (SMS) messages. Research staff enter baseline and secondary outcome data from the participant and infant medical notes. The trial aims to recruit a total of 132 participants. The trial design is summarised in Fig. 1.

Data will be collected at six timepoints, described in Fig. 2 and Table 2. At baseline, the participant completes a case report form (CRF) questionnaire about themselves, their previous breastmilk feeding experience and feeding intentions (B1a). Research staff complete a trial entry CRF with information about the birth and infant's medical status at randomisation (B1b). In the expressing phase of data collection, the participant completes a CRF questionnaire and 24-h log of expressed milk at three timepoints. E1 is day 4 after the infant's birth (the day of birth is termed day 0). E2 is day 14 and E3 is day 21 after the infant's birth. In the feeding phase of data collection, the participant responds to an SMS message asking about the infant's feeding status, at two timepoints. F1 is at 36 weeks' post-menstrual age (PMA; 4 weeks before the estimated date of delivery—the EDD—which defines 40

weeks of gestation) and F2 is at 18 weeks after the EDD—their SMS response automatically completes a CRF for each timepoint. If no SMS is received, infant feeding status can be extracted from the medical notes.

Two further exploratory data collection timepoints will take place, which do not contribute data to the RCT, but to exploratory analyses. These are a 24-h expressing log and questionnaire CRF at 32 weeks PMA, for participants who had given birth at less than 27 weeks PMA; and a feeding assessment SMS message at 9 weeks after the EDD. For example, an infant born at 24 weeks of gestation is 8 weeks old at 32 weeks PMA and 25 weeks old at 9 weeks after the EDD.

The trial forms part of a doctoral thesis. For reasons of efficiency within the available time, the recruitment period is divided into two sections. The majority of recruitment (10 months) will take place in the 'complete follow-up' period where participants will receive both F1 and F2 SMS messages. The final 22 weeks of recruitment will form the 'abbreviated follow-up' period where participants will receive only the F1 SMS message.

The time from recruitment to the end of the expressing phase of the study is 3 weeks. The time from recruitment to the end of the feeding phase of the study varies with the gestation of the infant and the follow-up period. The longest possible time—for a participant recruited in the 'complete follow-up' period with an infant born at 23 weeks—will be 35 weeks. The shortest possible time—for a participant recruited in the 'abbreviated follow-up' period with an infant born at 31 weeks and six days—will be 4 weeks. Figure 3 shows sample timelines for the most and least premature infants.

### Setting and participants

The trial plans to recruit participants within three National Healthcare Service Trusts in northern and southern England, covering four individual neonatal units (three tertiary level intensive care units and one local neonatal unit). All sites have at least one dedicated infant feeding support professional and counselling or psychological support is available. All sites have hospital-grade pumps available for use in the hospital and

**Table 1** Primary objective in PICO format

|                     | Primary objective                                                                                                              |
|---------------------|--------------------------------------------------------------------------------------------------------------------------------|
| <b>Population</b>   | Mothers of one or two infants born between 23+0 and 31+6 weeks' post-menstrual age                                             |
| <b>Intervention</b> | Provision of specific relaxation and visualisation recording with instruction to listen frequently while expressing breastmilk |
| <b>Control</b>      | Standard care, including standard lactation support provided by neonatal unit staff                                            |
| <b>Outcome</b>      | Expressed milk yield in 24 h (highest value recorded on any of day 4, day 14 or day 21 after birth)                            |

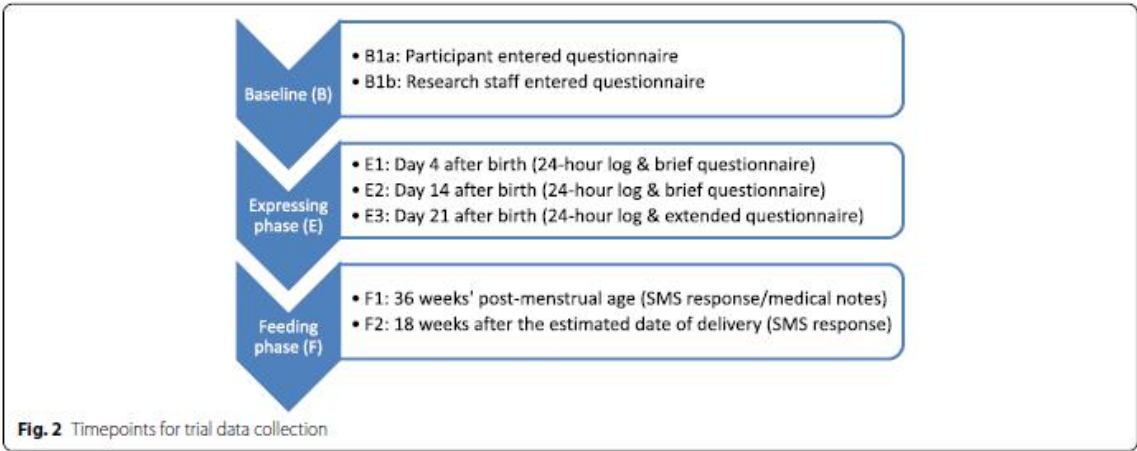
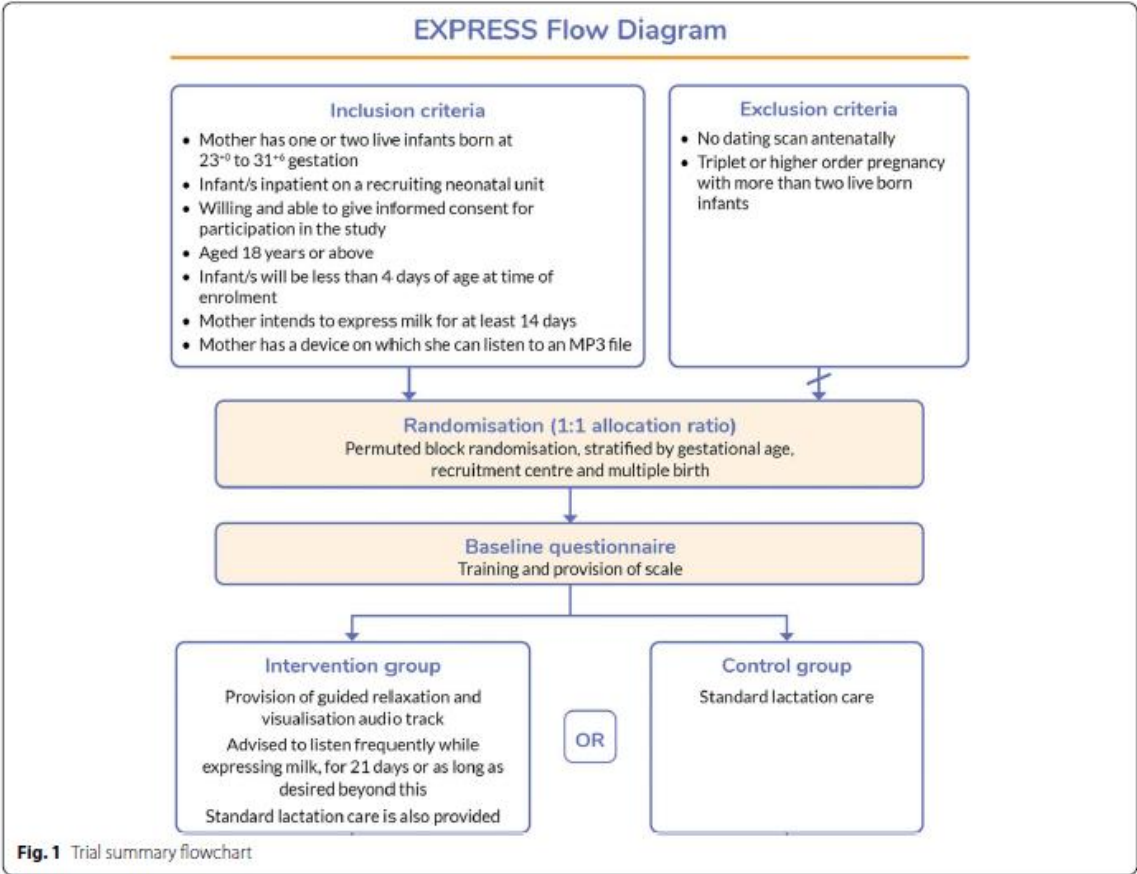


**Table 2** Schedule of trial procedures

| Procedures                                        | From the day of birth (day 0) up to midnight on day 3 of life | Day 4, 14 (E1 & E2) | Day 4 to 7 | Day 21 (E3) | 32 weeks post-menstrual age (exploratory timepoint)<br><i>Only applies if infant born at &lt;27 weeks gestation</i> | 36 weeks post-menstrual age (F1), 9 and 18 weeks after estimated date of delivery (F2)<br><i>In the abbreviated follow-up period the trial completes at F1</i> |
|---------------------------------------------------|---------------------------------------------------------------|---------------------|------------|-------------|---------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Informed consent                                  | ✓                                                             |                     |            |             |                                                                                                                     |                                                                                                                                                                |
| Eligibility assessment                            | ✓                                                             |                     |            |             |                                                                                                                     |                                                                                                                                                                |
| Randomisation                                     | ✓                                                             |                     |            |             |                                                                                                                     |                                                                                                                                                                |
| Baseline Questionnaire                            | ✓                                                             |                     |            |             |                                                                                                                     |                                                                                                                                                                |
| Maternal training and assessment on use of scales | ✓                                                             |                     |            |             |                                                                                                                     |                                                                                                                                                                |
| Check-in and verify accurate use of scales        |                                                               |                     | ✓          |             |                                                                                                                     |                                                                                                                                                                |
| Questionnaire & 24-h expressing log               |                                                               | ✓                   |            | ✓           |                                                                                                                     |                                                                                                                                                                |
| Maternal mental health questionnaires             |                                                               |                     |            | ✓           |                                                                                                                     |                                                                                                                                                                |
| Text message response                             |                                                               |                     |            |             | ✓                                                                                                                   | ✓                                                                                                                                                              |



a loan scheme for free use at home. Breastmilk feeding rates at discharge are variable, with one site below the regional and national averages and the others above average [42]. Two Trusts have level 3 Unicef Baby Friendly Initiative accreditation and one has not yet started the

accreditation process. All sites are urban and serve a range of communities, including diversity of ethnic origin and social deprivation.

Participants are mothers of infants born between 23<sup>+0</sup> and 31<sup>+6</sup> weeks of gestation. Full inclusion and exclusion



criteria are provided in Fig. 1. Participants with more than two live born infants are excluded and participants must have an intention to express milk for at least 2 weeks after birth to join the trial. Participants can be screened by research nurses and trained clinicians during pregnancy and after birth. Participants must provide written, informed consent (either on paper or electronically) and are provided with written information sheets. Randomisation can only take place between birth and midnight on day 3 after birth.

### Description of intervention

The intervention is the provision of a specific recording to the participant as an MP3 file with a request to listen frequently during expression of milk, on their own device, for at least 3 weeks. The intervention recording lasts approximately 12 min and consists of a guided relaxation and expression-specific visualisation. This is a modified version of an existing soundtrack used for previous studies [25, 30, 43, 44], modified and used under licence from the original author. The visualisation includes descriptions of pleasant surroundings, milk flowing in the breasts and skin-to-skin contact with the infant. The modified version will be made freely available after the trial is complete.

Both intervention and control groups will receive general lactation care, including routine advice from neonatal unit staff and standardised printed information on milk expression complying with Unicef Baby Friendly Initiative recommendations [45].

### Randomisation

Participants will be randomised with a 1:1 allocation using stratified permuted block randomisation by a secure web-based randomisation programme hosted by the National Perinatal Epidemiology Unit Clinical Trials Unit. Stratification will be based on recruitment neonatal unit, gestational age at birth ( $23^{+0}$  to  $27^{+6}$  weeks versus  $28^{+0}$  to  $31^{+6}$  weeks) and multiple birth (one versus two babies alive at time of randomisation). Research staff and the participant are aware of the allocation after randomisation, as the study is unmasked. Study identification numbers are not consecutive, to reduce the possibility of research staff monitoring the sequence of allocation.

### Primary outcome

The primary outcome is defined as the highest 24-h expressed milk weight recorded on any of day 4, day 14 or day 21 (E1, E2 or E3; in grams). If the participant declares that they are no longer expressing, the milk weight for that day is accorded a value of zero. Table 3 shows various scenarios of what this means in practice. The reason for this definition of the primary outcome, instead of

**Table 3** Examples of primary outcome calculation

|            | Day 4 | Day 14  | Day 21                  | Primary outcome |
|------------|-------|---------|-------------------------|-----------------|
| Scenario 1 | 300g  | 500g    | 750g                    | 750g            |
| Scenario 2 | 100g  | 450g    | 400g                    | 450g            |
| Scenario 3 | 50g   | missing | missing                 | 50g             |
| Scenario 4 | 50g   | 150g    | Stopped expressing (0g) | 150g            |

using 24-h expressed milk weight at a specific timepoint such as day 21, is that some more mature infants may start to directly breastfeed by day 21 and this is likely to decrease expressed milk yield. In addition, some parents may choose to stop expressing before day 21 but increasing their milk yield prior to this would still be an important outcome to reduce infant morbidity. Finally, the physiology of lactation studied in term infants [46] suggests that once a milk supply is established, it is robust to supply and demand—therefore, achieving a higher milk yield at day 14 than day 21 suggests that the parent will be able to increase their yield as needed with more frequent expressing in the future.

### Secondary outcomes

The following secondary outcomes will be reported:

- Proportion of participants expressing at least 750g of milk in 24 h on any of day 4, 14 or 21 (E1, E2 or E3)
- Rate of milk expression (in grams per minute) at day 21 (E3)
- Average Spielberger State Trait Anxiety Index (six-item format; STAI-6) score at day 21 (E3)
- Average Post-traumatic Stress Checklist for DSM-5 (PCL-5) score at day 21 (E3)
- Proportion of participants feeding their infant/s only breastmilk (no infant formula) at 36 weeks PMA (F1)
- Proportion of participants feeding their infant/s any breastmilk at 36 weeks PMA (F1)
- Proportion of participants feeding their infant/s only breastmilk (no infant formula) at 18 weeks after the estimated date of delivery (4 months corrected age—F2)

The STAI is a reliable measure of anxiety [47] and widely used, including in studies after very premature birth [48, 49] and after use of a relaxation intervention in the neonatal intensive care unit [27]. The original STAI is a 20-item questionnaire with four answer options for each question ('not at all', 'somewhat', 'moderately' or 'very much'). The total score range is 20–80, with higher scores signifying more anxiety, <36

generally considered normal [50] and  $\geq 40$  signifying clinically significant anxiety [48] (although this cut off varies across the literature [5]). The shortened STAI-6 contains a subset of six questions, giving a score total of 6–24, which are then scaled to 20–80 for comparability [51]. The short form is highly correlated with the 20-item STAI, with internal consistency greater than 0.9 [52]. The minimal meaningful difference in STAI score has been suggested as 10 [53].

The PCL-5 is a commonly used [54] publicly available screening assessment for post-traumatic stress disorder (PTSD) aligned to the Diagnostic and Statistical Manual of Mental Disorders-Fifth Edition (DSM-5) [55]. It has been used in the context of birth related trauma [56]. The PCL-5 is very similar to the Impact of Event Scale (IES) and the preceding PCL for DSM-IV that have both been used in the specific context of premature birth [48, 57]. The PCL-5 [55] has 20 items with four answer options for each question ('not at all', 'a little bit', 'moderately', 'quite a bit' and 'extremely'). The total score range is 0–80, with higher scores signifying more distress. A cut off of 31–33 [58, 59] has been proposed as indicative for probable PTSD. A 5–10 point change is likely to be the minimum threshold for a clinically meaningful difference [55]. The PCL-5 has internal consistency (for example, Cronbach's alpha 0.95) and good construct validity in a variety of settings [58–60]. Of note, a diagnosis of PTSD requires symptoms to continue beyond 28 days after the traumatic event—therefore, in this context, the PCL-5 will measure post-traumatic stress reactions rather than PTSD.

In relation to the secondary outcomes measured at 36 weeks PMA and 4 months corrected age (F1 and F2), note that no reference is made to whether the infant is having complementary feeds ('solids') in addition to milk. This is because the timing of introduction of complementary feeds for premature babies is not recommended by actual age as it is with term babies, but rather personalised to the developmental stage, actual and corrected age of the infant [61].

#### Process indicators

The following process indicators will be reported:

- Time spent in skin to skin contact with infant/s at day 21 (E3; in hours)
- Number of expressing episodes in 24 h at day 21 (E3)
- Total time spent expressing in 24 h at day 21 (E3; in hours)

The process indicators were chosen as they may indicate a behavioural pathway for any effect seen on the primary outcome.

#### Monitoring adherence and contamination

At E1, E2 and E3, participants in the intervention group are asked how many times they have listened to the intervention in the previous 24 h. At E3, both groups are asked how often they have practised other forms of relaxation since the birth of their infant/s.

#### Measures to improve data quality and retention

Research nurses and trained clinicians will have close contact with participants during the expressing phase. At baseline, participants are given written information on weighing milk and using the electronic forms; short videos are also available. Research staff contact participants after the first expressing log (E1) to ensure that the weighing record is accurate and respond to questions. Automated reminder emails and SMS messages are sent to participants if forms are not filled in. Trial staff check participant-submitted forms and research staff raise any obvious problems with participants in a timely period. Automated SMS messages are sent to the intervention group on day 9 and day 17 after birth to remind them to listen to the intervention. Prior to the feeding phase assessments (F1 and F2), emails are sent explaining their importance.

#### Withdrawal and change in consent

Each participant has the right to change their consent at any time and will not be contacted for further data submission after this time. In addition, the Investigator may withdraw a participant from the study at any time if the Investigator considers it necessary for any reason including:

- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Death of the mother or the infant/s during the study period

The number of participants withdrawn and changing their consent status will be reported by trial arm, with reasons. Participants choosing to stop listening to the intervention recording but continuing follow-up will remain in the study. Participants may choose to continue in the study after the death of a single twin.

#### Data collection

At E1, E2 and E3, participants will be asked to record information about each time that they attempt to express milk in a 24-h period, starting when they wake up for the day. Participants will be trained at recruitment to weigh their expressed milk inside its container and to record the weight of the empty container. At



analysis, the pure milk weight expressed can therefore be calculated and summed over the 24-h period. Each participant will be given a portable digital scale accurate to 0.1g (Kabalo). Participants can fill in the expressing log and questionnaire on paper or on an electronic form with individual URL, hosted by OpenClinica (a third party application hosted in the UK by an ISO 27001:2013 accredited third party; AA). Paper forms can be sent by post or photographs can be uploaded securely by participants. Participants will receive automated notifications the night before and on the morning of the scheduled day, and a reminder the next day if appropriate. The scheduled period for data response is within 48 h of the scheduled day.

Participants can express milk by whatever method they desire. By day 4 after birth (E1), all sites recommend participants to use a double hospital-grade electric pump.

At F1 and F2, participants are sent an SMS message asking what type of milk their infant/s is drinking—only breastmilk, only infant formula or a mixture of the two. The scheduled period for data response is within 7 days of the respective timepoints. At F1, infant feeding status can be extracted from medical notes if no response is received.

Basic demographics of the potentially eligible population will be extracted from routinely entered clinical data in the BadgerNet platform by clinical staff and fully anonymised before transfer to the NPEU, as a screening log.

No formal safety reporting is required due to the low risk nature of the intervention.

### Project management

The trial will be run on a day-to-day basis by the Project Management Group (PMG), which reports to the Trial Steering Committee (TSC). The TSC receives recommendations related to the data arising from the trial from the Data Monitoring Committee (DMC). The PMG will consist of the Chief Investigator, Clinical Trials Unit Director, Head of Operations, Senior Trials Manager, Trial Programmers, the Trial Statistician and Trial Administrator. The PMG will meet every 3 to 4 weeks. The TSC and DMC will consist of independent clinical and statistical experts, as well as parent and public representatives. The TSC and DMC will meet three times over the course of the trial; charters are available by contacting the study team. Both are independent from the sponsor. The trial will be run according to the Clinical Trials Unit standard operating procedures, including potential audit. The sponsor is the University of Oxford ([ctr@admin.ox.ac.uk](mailto:ctr@admin.ox.ac.uk)), which has ultimate authority over the trial's scientific integrity.

### Patient and public involvement

National charity for sick and preterm babies, Bliss, has been a co-applicant for the study since its inception. A total of 675 people who had experience of premature birth were involved in initial online engagement work to define the most important outcomes for the RCT, give their thoughts on ethical issues and share the ways in which they found information about lactation to inform the dissemination plan. From this pool of parents, six also attended virtual panels to give detailed feedback on trial design and participant-facing documents, and a further 12 took part in an exercise to advise on modifications to the intervention recording. Modifications were made to the intervention recording in three key areas raised by parents—minimising a feeling of pressure on volumes of milk expressed, sensitive language related to potentially traumatic birth and language appropriate to immature and potentially sick infants. Bliss and a parent contributor are members of the Trial Steering Committee.

### Statistics and analysis

#### Sample size and power calculation

In the most relevant previous study [25], mean expressed milk yield at day 14 of the trial increased from 318 ml (standard deviation; SD 309 ml) to 862 ml (SD 309 ml) with the use of a visualisation/relaxation soundtrack. Initial exploratory work suggested that baseline milk yield in the recruiting sites is higher than this, and therefore, this study is powered to detect an increase in highest 24-h expressed milk yield from 670g (SD 300g) to 825g (SD 300g), with 80% power and a two-sided significance level of 0.05. This is a 155g absolute mean difference or a 0.5 standardised mean difference. In total, 118 participants are required, so the recruitment target was set at 132 to allow for 10% attrition. Since breastmilk has an average specific gravity of 1.03 [62, 63], 670g is estimated as equivalent to 650ml and 825g is estimated as equivalent to 800ml, although much of the literature views volume and mass measurements as interchangeable for breastmilk [64–66].

#### Description of statistical methods

Demographic and clinical data will be summarised with counts and percentages for categorical variables, means (with SDs) for normally distributed continuous variables and medians (with interquartile or simple ranges) for other continuous variables.

Statistical analysis of continuous outcomes will use linear regression with adjusted and unadjusted mean differences presented, or quantile regression with median differences presented, as appropriate. Binary outcomes will be analysed using log binomial regression, or Poisson

regression with a robust variance estimator if the model fails to converge, and risk ratios will be presented. 95% confidence intervals will be presented.

Analyses will be adjusted for the stratification factors (recruiting neonatal unit, gestational age at birth and whether the participant has one or two babies alive at randomisation) where possible. The STAI score at day 21 will be adjusted for the STAI score at baseline. The primary outcome will be adjusted for the associated measurement day of the highest milk weight. Both crude and adjusted estimates will be presented, but the primary inference will be based on the adjusted estimates. Further process indicators, such as number of direct breastfeeds, will be summarised by randomised arm, with no comparative statistics presented.

Participants will be analysed in the groups to which they were randomly assigned, comparing the outcome of all participants allocated to intervention with all those allocated to the comparator group, regardless of deviation from the protocol or treatment received (the Intention to Treat population). Analysis will take place on a superiority basis.

Exploratory subgroup analysis will use a statistical test of interaction to examine the effect of the intervention on the primary outcome by gestational age at birth (<28 weeks and  $\geq 28$  weeks). Stratified analysis by the other stratification factors described and exclusive breastmilk feeding intention at baseline will take place with no formal test for interaction. This is to limit multiple comparisons and due to the low statistical power for subgroup analysis.

An exploration of adherence effect on the primary outcome will be conducted, dividing the intervention group into high- and low-frequency adherence by their reported number of times listening to the recording. An exploration of perceived relaxation effect on primary outcome will be conducted, dividing the intervention group into those who report the intervention to be relaxing and those who do not (via Likert scale). Summary statistics will be presented, with no statistical inference.

Basic demographics of the recruited participants will be compared with aggregate data from the potentially eligible population with no statistical inferences made.

Primary analysis will take place on a complete case basis. If more than 10% of primary outcome data is missing, sensitivity analysis will be performed to explore the pattern of missingness for the primary outcome, using multiple imputation and/or a pattern mixture model as appropriate. Sensitivity analyses will also assess whether the definition of the primary outcome has led to bias by assessing the impact of using day 21 expressing yield only, and separately of excluding day 4 logs. Stata will be used for all analysis.

#### **Participant confidentiality and retention of personal data**

The Participant Information Sheet provides the required information about data processing. Participant contact details are necessary to send CRFs and SMS messages. These will be kept in an administrative database, linked to the clinical database by study identifier. All electronic databases comply with relevant security standards and are regularly backed up. Identifiable information will be kept centrally for a period of 5 years and then reviewed. All data kept centrally will be archived according to Clinical Trials Unit standard operating procedures.

#### **Participant remuneration**

Each participant will be provided with a small digital weighing scale, a coolbag and a pen. They will be offered a set of headphones if in the intervention group. Due to higher than expected loss to follow-up for the primary outcome, an amendment was approved after recruitment started to provide a £10 electronic food and drink voucher code to participants during the expressing phase. This is an unconditional thank you gift for the participant with the aim to increase the participant's feeling of connection with and positivity about the trial.

#### **SPIRIT guidelines**

This protocol report uses the SPIRIT reporting guidelines [67]. The SPIRIT checklist is provided as supplementary material (Additional file 1).

#### **Discussion**

##### **Ethical considerations**

The trial will run in the context of families experiencing the trauma of very premature birth. Many babies will be very unwell, or perceived to be very unwell by their families even when their clinical course is smooth. Some babies may die, including one of a multiple birth where the other baby survives. Much of the recruitment period will take place at a time when parents are likely to be further affected by the Covid-19 pandemic, both in their lives outside of the neonatal unit and in terms of potential increased separation from and anxiety about their baby. The potential participants of the trial are therefore facing many ethical crises and are under major stress throughout the period of the trial. The trial team has collaborated with parent panels and national charity for sick and premature babies, Bliss, to ensure that the trial structure and the language of all trial documents take this situation into account. All participant-facing CRFs include links for more information about having a premature baby and expressing milk. Free text questions are included to allow participants to express their feelings and feel seen as individuals. Infant deaths will be frequently monitored during the trial period to ensure we are not asking mothers



for research data at the time of their bereavement. Staff will be encouraged to undergo bereavement training.

If the participants' scores on the mental health assessments at day 21 exceed a pre-specified threshold for concern, the participant will be sent information on self-help and how to access individualised neonatal unit-based psychological support.

#### Dissemination

A dissemination plan has been developed, using the parent involvement process to identify key sources of information about lactation. The trial findings will be disseminated via academic conferences and publications, to clinical staff via clinical networks and to parents via social media and online support networks as well as printed information distributed to neonatal units. There are no plans to use professional writers and all authors will fulfil authorship criteria.

#### Impact and risk of bias

This study will report on whether a simple, easily disseminated relaxation intervention improves expressed milk yield, duration of breastmilk feeding and mental health outcomes for mothers of very premature infants. The intervention recording will be made freely available after the results are reported, so if proven beneficial, there will be an immediate positive impact on parents globally, with the caveat of the English language nature of the recording. A strength of this trial is the robust design with full Clinical Trials Unit support, which is unusual for RCTs in the field of lactation. Further strengths include extensive parent involvement, a nuanced clinically important primary outcome and pre-specified sensitivity analyses to assess potential bias.

Study outcomes will be broadly generalisable to parents of infants born at less than 32 weeks of gestational age in countries with advanced neonatal care, and are likely to apply to parents expressing milk for sick infants of all gestations as well. Inclusion criteria are broad to recruit a representative population, not just those who are highly motivated to express and exclusively breastfeed, which increases external validity. It is possible that the effect of the intervention will depend on the level of wider support available to the participants and the trial includes neonatal units with a range of lactation support intensity.

There is likely to be some 'trial effect' reducing external validity—it is known that the use of expressing logs and increased focus on expressing characteristics can improve adherence to recommendations and therefore may improve outcomes across all participants, including the control group [68]. However, there is no way to overcome these limitations while robustly studying expression characteristics.

There is inherent potential for bias in all trials where the intervention cannot by its nature be blinded. Participants in the intervention group may express more frequently or for longer periods of time in order to please the research team or because they believe in the power of the intervention. The study is designed to report process indicators to see if this has taken place (although these factors are also possible behavioural mechanisms for an intervention affect, rather than necessarily a sign of bias). Techniques of partial blinding due to deception used in other trials [30, 43, 44] (where the control group are not aware of the existence of the intervention) are not appropriate where participants interact with each other frequently. It is therefore acknowledged that the study will have some unmodifiable risk of bias. Nevertheless, it will be a worthwhile contribution to understanding lactation in challenging circumstances.

#### Trial status

Recruitment began 2 August 2021. Recruitment completion will be 31 October 2022. Current protocol is version 5.0 26/05/2022. Amendments are submitted to the Research Ethics Committee and then disseminated as recommended by the committee.

#### Abbreviations

CRF: Case report form; DMC: Data Monitoring Committee; DSM: Diagnostic and Statistical Manual of Mental Disorders; EDD: Estimated date of delivery; MER: Milk ejection reflex; PCL: Post-traumatic Stress Checklist; PMA: Post-menstrual age; PMG: Project Management Group; PTSD: Post-traumatic stress disorder; RCT: Randomised controlled trial; SD: Standard deviation; SMS: Short messaging service; STAI: Spielberger State Trait Anxiety Index; TSC: Trial Steering Committee.

#### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13063-022-06570-9>.

**Additional file 1.** SPIRIT Checklist. Completed SPIRIT Checklist as per journal requirement.

#### Acknowledgements

The authors are very grateful to Bliss and all the parents who contributed to trial planning and design. Richard Welsh, Andy King and David Murray contributed to the design of the IT aspects of the trial. Sarah Chamberlain and Andy Kirk contributed to the graphic design and website for the trial. TSC and DMC members gave feedback on the protocol before finalisation.

#### Authors' contributions

IL is the Chief Investigator; she conceived the study, led the proposal and protocol development. JLB was the trial statistician at time of protocol creation; she participated in protocol creation. CC and KS are senior members of the Clinical Trials Unit leading the trial; they participated in the creation of the protocol and ongoing management of the trial. FOB is IL's doctoral supervisor; she participated in protocol creation and ongoing management of the trial. MF is IL's doctoral supervisor; she participated in protocol creation. MQ is IL's primary doctoral supervisor; she participated in protocol creation and ongoing management of the trial. All authors read and approved the final manuscript.

### Authors' Information

IL is chair of the Hospital Infant Feeding Network, a knowledge-sharing organisation for hospital professionals interested in infant feeding (voluntary position). IL is a trustee of a community charity, Oxfordshire Breastfeeding Support (voluntary position).

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### Availability of data and materials

The authors welcome requests from researchers to access data held by the Nuffield Department of Population Health (NDPH). Access to the datasets will be controlled by the data custodian and subject to further regulatory approvals. The NDPH Data Access policy sets out the criteria and process for data sharing. Requests for data sharing that meet the criteria will be considered by the NDPH data sharing committee. The criteria are as follows: to ensure the integrity of any data that it releases to the public (e.g. resolving data quality issues); and to allow a reasonable period of exclusivity. The committee will assure themselves that the proposal is scientifically sound, the protocol has been peer reviewed, there is adequate funding and there are appropriate ethics approvals in place. The committee will require evidence that the likely commitment of NDPH staff has been realistically assessed and will normally expect funding to cover any NDPH costs in making data available and providing ongoing support. The committee must also assure itself that releasing the data can be justified within the scope of the original participant consent. Shared data will be anonymous. The following information is given in the Participant Information Sheet for this study: 'Anonymised information from this study may be shared with other researchers doing similar research in the future. None of your personal identifiable information will be shared, and you will not be identifiable from this data'.

### Declarations

#### Ethics approval and consent to participate

Bloomsbury Research Ethics Committee 21/LC/0279. Written, informed consent to participate will be obtained from all participants.

#### Consent for publication

Willing to provide a model consent form, participant information sheet and CRFs on request.

#### Competing interests

MF receives an unrestricted donation for research on infant nutrition from Philips (not related to the current trial). The remaining authors declare that they have no competing interests. No principal investigators at study sites have declared any competing interests.

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## Appendix D: Excerpts from the Statistical Analysis Plan for the randomised trial (related to Chapter 5)

### Background

The expressing log that participants are requested to fill out at each timepoint, either on paper or electronically, is shown here:

### 24 hour expressing log

Please log each time you express milk today, starting from when you wake up for the day and continuing for 24 hours after the first time you express (for example, if you first express at 09:30 please log every time you express until 09:29 the following day).

If you are no longer expressing milk, please tick this box ☐

If you plan to continue expressing milk but you have not expressed at all in this 24 hour period, please tick this box ☐

If you are using the standard expressing containers from John Radcliffe Hospital neonatal unit you can use the following weights when the log asks for the weight of the empty container. Large 130ml bottle (with lid) = 22g. Medium 100ml bottle (with lid) = 18.5g. Colostrum syringe (with cap) = 4g. If you are using a different container, including if you move to a different hospital with different bottles, then please weigh it when it's empty.

| When did you start expressing?                                     |                                                                    | Method                   |                          |                          | How long did you express for? (minutes) | Weight of milk and container - right breast (with lid on; grams) | Weight of milk and container - left breast (with lid on; grams) | Weight of empty container (with lid on; grams) |
|--------------------------------------------------------------------|--------------------------------------------------------------------|--------------------------|--------------------------|--------------------------|-----------------------------------------|------------------------------------------------------------------|-----------------------------------------------------------------|------------------------------------------------|
| Date                                                               | Time                                                               | Hand                     | Manual pump              | Electric pump            |                                         |                                                                  |                                                                 |                                                |
| <input type="text"/> / <input type="text"/> / <input type="text"/> | <input type="text"/> : <input type="text"/> : <input type="text"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="text"/>                    | <input type="text"/> + <input type="text"/>                      | <input type="text"/> + <input type="text"/>                     | <input type="text"/> + <input type="text"/>    |
| <input type="text"/> / <input type="text"/> / <input type="text"/> | <input type="text"/> : <input type="text"/> : <input type="text"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="text"/>                    | <input type="text"/> + <input type="text"/>                      | <input type="text"/> + <input type="text"/>                     | <input type="text"/> + <input type="text"/>    |

| When did you start expressing?          |                                          |                                                                                                                      |                                                 |
|-----------------------------------------|------------------------------------------|----------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|
| Date                                    | Time (hh:mm)                             | Method<br>Please select all that apply                                                                               |                                                 |
| 2021-12-22                              | 05:00                                    | <input type="checkbox"/> Hand <input type="checkbox"/> Manual pump <input checked="" type="checkbox"/> Electric pump |                                                 |
| How long did you express for? (minutes) | Weight of milk from right breast (grams) | Weight of milk from left breast (grams)                                                                              | Weight of empty container (with lid on) (grams) |
| 20                                      | 34.7                                     | 38.9                                                                                                                 | 18                                              |

+

This shows that each time the participant expresses milk they are asked to record the date and time that they started, the expressing method, the number of minutes they spent expressing and the weight of milk (and its container) produced by each breast. If they express a single breast then they provide a single weight of milk (and its container). If they express both breasts simultaneously (the recommended method) then they provide two weights of milk, each including the container (the container is assumed to be the same weight for both breasts).



The final piece of information recorded is the weight of the empty container so that pure milk weights can be calculated at the analysis stage. If the participant is using standard containers provided by the recruiting neonatal unit then the weights are provided in text format on the log.

#### **Data monitoring, interim analyses and stopping rules**

The Data Monitoring Committee (DMC) will consider accumulating information relating to safety, recruitment and data quality (e.g. data return rates, treatment adherence, protocol adherence, withdrawal). Unblinded group data will be available to the DMC to assess baseline comparability, differential withdrawal/data quality and assumptions used in powering the study but no statistical tests will be performed due to the size of the trial and there are no pre-specified stopping rules. An interim meeting will occur after six to nine months of trial recruitment. A report will be prepared by an independent trial statistician.

#### **Trial reporting**

The trial will be reported according to the principles of the CONSORT statement. Data lock will occur in two stages – first data lock will occur once data entry is complete and all queries have been resolved related to the period up to and including day 21 forms for the last participant recruited (PQ3). Final data lock will occur once data entry is complete and all queries have been resolved related to the final chronological timepoint for all participants (OQ1 for those recruited in the ‘abbreviated follow up’ period and OQ3 for those recruited in the ‘complete follow up’ period)

#### **Protocol non-compliances**

A protocol non-compliance is defined as a failure to adhere to the protocol such as the wrong allocation being administered, incorrect data being collected and documented, errors in applying inclusion/exclusion criteria or missed follow-up visits due to error.

All protocol non-compliances will be listed in the final report. Non-compliances are defined below.

## Major

The following are pre-defined major protocol non-compliances with a direct bearing on the primary outcome:

- Data considered to be fraudulent

## Minor

The following will be defined as minor protocol non-compliances:

- Participants randomised in error. These include participants:
  - With infants born < 23 weeks' or ≥ 32 weeks' PMA
  - With infants ≥ 4 days old
  - With more than two live infants
  - Aged under 18 years
  - With no antenatal dating scan
  - Where written informed consent has not been obtained
- Treatment non-compliances. These include participants who:
  - Are in the recording intervention group and do not receive the study recording
  - Are in the recording intervention group and receive the study recording more than 4 days after birth
  - Are in the control group and receive the study recording
- Trial procedure non-compliances:
  - Trial Entry Form not completed by site staff
  - Day 4-7 check in not completed by site staff within scheduled window
  - Participant entered data not completed within scheduled windows
  - Are in the recording intervention group and never listen to the study recording
  - Are in the control group and regularly listen to or practice independently sourced guided relaxation material

Protocol non-compliances will be reported in a flowchart and process outcomes table.

## **Analysis populations**

### **Post-randomisation exclusions**

Exclusions to the analysis post-randomisation are defined as any of the following:

- Participants for whom a written consent form was not received
- Participants whose consent to use their data was withdrawn (excluded from the point of withdrawal)
- Participants for whom an entire record of fraudulent data was detected (should fraudulent data be detected, consideration will be given to excluding all data for the site where such data were found).

The numbers and proportion of post-randomisation exclusions will be reported by randomised treatment group, and reasons summarised.

### **Population definitions**

The intention to treat (ITT) population will be all participants randomised, excluding post-randomisation exclusions. Note that the population assessed for the final trial outcome (exclusive HMF at 18 weeks after the EDD) is formed of only the participants recruited in the complete follow up period, not the abbreviated follow up period.

In the interim analysis, baseline and outcome data will be reported for all trial participants with available data, excluding known post-randomisation exclusions.

## **Descriptive analyses**

### **Representativeness of trial population and participant throughput**

A flow diagram will be reported showing number of participants randomised, allocated and providing data at each timepoint, as well as number providing data for the primary outcome analysis. Post-randomisation exclusions and specifically a change of consent (withdrawal) due to infant/s death and other reasons will be presented. Number of participants in the complete follow up period and the abbreviated follow up period will be reported. Measurements of recording intervention adherence and

contamination will be reported in table format (number of participants in recording intervention group reporting never listening to recording on day 4 or day 14 or day 21 questionnaires; number of participants reporting listening to/practising other relaxation material in trial period at day 21). Reporting will follow CONSORT guidance.

### **Baseline comparability of randomised groups**

The intention to treat analysis population will be used and all baseline characteristics reported in table format.

The following maternal characteristics will be described in the baseline tables:

- Ethnicity
- Age at randomisation
- Deprivation index quintile
- Age at leaving full time education
- Living with a partner
- Current smoker
- Admitted to ITU in first 48 hours after birth
- Time from birth to first expression of milk
- Intention for exclusive HMF at the time of discharge
- STAI-6 score
- Mode of delivery
- Parity
- Previous HMF experience (binary and length of longest previous HMF)

The following infant characteristics will be described in the baseline tables:

- Recruiting centre at randomisation
- Place of birth (randomisation centre or not)
- Gestational age at birth
- Age at randomisation

- Single or twin birth
- Apgar score at five minutes
- Ventilated at randomisation

If a participant has two babies, the most invasive birth method, the most invasive respiratory support and the lowest Apgar score of the two babies is reported.

Categorical data will be presented using counts and percentages, continuous data will be presented using number of participants, mean, median, standard deviation, minimum, maximum and/or interquartile range. There will be no tests of statistical significance performed nor confidence intervals calculated for differences between randomised groups on any baseline variable.

STAI-6 score at baseline will in addition be presented as number and percentage of participants with score >40. Time after birth at randomisation will be presented by group. Participant postcode is transformed to quintiles of index of multiple deprivation using publicly available calculators.

#### **Losses to follow-up**

Change of consent (withdrawal) and loss to follow up will be reported in the flowchart as mentioned above. A moderate level of loss to follow up is expected as the study relies on participant reported outcomes at a time of high stress. Infant mortality of 10% is expected, with associated withdrawal – although part of the impact is expected to fall after data has been contributed for the primary outcome.

Any baseline information collected on losses to follow-up may be compared with those who completed the trial to the primary outcome and presented using summary statistics.

#### **Adherence to recording intervention**

Adherence with the recording intervention will be reported in the process indicator tables (number of times listened to the study recording in previous 24 hours at each log timepoint). An assessment of whether participants in both arms have practiced non-intervention relaxation in the study period as assessed at day 21 is also reported.

#### **Comparative analyses**

## Detailed definition of primary outcome

### ***Primary outcome: 24-hour weight of expressed milk***

The following is a step by step description of how data is extracted from the participant log to derive the 24-hour weight:

The pure milk weight expressed at each session is calculated by subtracting the weight of the empty container from each combined milk/container weight recorded.

If this pure milk weight is negative (container is recorded as heavier than the combined milk/container weight) then the value is classified as erroneous data. However if a single combined milk/container weight is recorded as zero then the pure milk weight will be classified as missing data instead.

If there is no container weight recorded then the value is classified as erroneous data

The 24-hour period is defined as the 24 hours following the start time of the first chronologically recorded log. Expressing sessions are included in the 24-hour yield if they are completed (defined by the end time of the expressing session) in  $\leq 24$  hours from the start time

The 24-hour yield at each timepoint is calculated by summing all non-erroneous milk weights in the 24-hour period

Examples of defining the 24-hour period:

| Potential scenario                                                                                  | Analysis impact                                              |
|-----------------------------------------------------------------------------------------------------|--------------------------------------------------------------|
| Participant starts expressing at 10am 12/02/2022. Final log recorded finishes at 10am 13/02/2022    | Milk expressed at final log is included in 24-hour total     |
| Participant starts expressing at 10am 12/02/2022. Final log recorded finishes at 10:01am 13/02/2022 | Milk expressed at final log is not included in 24-hour total |

Examples of defining erroneous data:

| Potential scenario                                                                                               | Analysis impact                                                        |
|------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|
| Participant records a single expressing session [milk + container] weight as 10g and the container weight as 13g | The milk weight for this session is -3g and is classified as erroneous |
| Participant does not submit the container weight for an expressing session                                       | The milk weight for this session is classified as erroneous            |

|                                                                                                                                                                                                                                      |                                                                                                                         |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|
| Participant records a valid weight for the right breast [milk + container] and records 0 for the left breast (perhaps as they have not attempted to express the left breast or have combined milk from both breasts into one bottle) | Zero is treated as missing data, not erroneous data. The milk weight for this session contributes to the 24 hour yield. |
| Participant records 0 [milk + container] weights for both breasts                                                                                                                                                                    | The milk weight for this session is classified as erroneous                                                             |

If a participant has reported that no attempt to express milk has been made in the 24-hour period, this will contribute a value of 0g as the 24-hour weight. If a participant has not provided a log, the value is missing.

After the 24-hour milk yield has been calculated for each timepoint, the primary outcome is identified as the highest of these values as noted above. Further detail is shown here:

| Potential scenario                                                                                            | Analysis impact                                                                                                                                |
|---------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| Three logs have been submitted (optimal scenario)                                                             | Primary outcome is the highest 24-hour total of the submitted logs                                                                             |
| Participant does not submit a log at one or two timepoints                                                    | 24-hour total is recorded as missing for any timepoints where a log is not submitted. The primary outcome is the highest of the submitted logs |
| Participant does not submit a log at any timepoint                                                            | Primary outcome is missing                                                                                                                     |
| Participant reports that they have not attempted to express milk in the 24-hour period for a log              | 24-hour total is 0g for this log                                                                                                               |
| Two logs have been submitted and one reported as no attempt to express milk in the 24-hour period             | Primary outcome is the highest 24-hour total of the submitted logs, including the log with 0g 24-hour total                                    |
| One or two logs are recorded as no attempt to express milk in the 24-hour period and the other log is missing | Primary outcome is 0g                                                                                                                          |
| All logs are recorded as no attempt to express milk in the 24-hour period                                     | Primary outcome is 0g                                                                                                                          |

Details of which timepoint forms the primary outcome will be presented by group with no statistical test, as well as details of which timepoints were available (non-missing) for the assessment of the primary outcome.

Logs will be included in the analysis for the scheduled timepoint regardless of the date of data that they refer to. For example if a participant submits a day 4 log and the data refers to day 7, it will be included in the analysis as a day 4 log. The number of logs falling outside the scheduled 48-hour window for each timepoint will be presented.

10am is used as an arbitrary scheduled start time for each logging timepoint to define the 48-hour window (for example a day 4 log will fall outside the scheduled window if the first logged expressing session starts before 10am on day 6).

Some participants unexpectedly expressed both breasts sequentially into the same container. This poses a problem to the format of the expressing log as milk weight is requested inside a separate container for each breast. Where this was noted by the researchers or raised by the participant, the participant was advised to allocate this milk/container weight to a single breast. No further analysis of milk weight by breast is used because of this potential inaccuracy in milk assigned to a specific breast. This does not affect the primary outcome.

Some participants unexpectedly expressed such a high quantity of milk that they required more than one bottle for each breast at a single expressing session. This poses a problem to the format of the expressing log as milk weight is requested as a single container of milk for each breast for each session. Where this was noted by the researchers or raised by the participant, the participant was advised to record multiple sessions with precise, non-overlapping start time and duration to match the time spent expressing milk into each set of containers. However in some cases participants logged multiple sessions with identical start time and duration, or with overlapping times and duration, or with zero duration, to deal with this scenario. This does not affect the primary outcome.

#### **Detailed definition of secondary outcomes**

##### ***Secondary outcome: Proportion expressing at least 750g milk in 24 hours***

The highest 24-hour milk weight forming the primary outcome is used to define whether the participant has expressed  $\geq 750\text{g}$  in any of the recorded 24-hour periods.

##### ***Secondary outcome: Average volume of milk expressed per minute***

The 24-hour milk weight recorded at day 21 is divided by the number of minutes spent expressing in 24 hours at day 21 to form this outcome.

As noted above, there are some scenarios which affect the calculation of an accurate milk expression rate – these are when erroneous data have been identified and where participants have used more than



one container for each breast during a single expressing session and may have logged this in a variety of ways. To ensure that the appropriate numerator and denominator are used in these cases, the following exclusions apply for this outcome:

Where an expressing session includes erroneous data then the whole session is excluded from the numerator (any milk weight associated with the session) and the denominator (any time associated with this session)

If both start time and duration for multiple logged sessions are identical then the duration of duplicate sessions will be excluded from the denominator

***Secondary outcome: Proportion with exclusive human milk feeding***

This outcome is assessed at two separate timepoints – 36 weeks' PMA and 18 weeks after the EDD. Exclusivity of human milk feeding will be defined as no intake of infant formula in the reference period (the 24 hours prior to the question being asked). If a participant has two babies, the outcome will be classified with reference to both infants (no infant formula given to either infant). Of note, intake of complementary food is not considered in this outcome. Participants respond to an SMS message to provide this information. At 36 weeks', this information can also be derived from the infant's medical notes where an SMS response is not received.

SMS responses will be included in the analysis for the scheduled timepoint regardless of the date that they are sent. For example, if a participant responds to the 36 week PMA SMS at 38 weeks PMA, it will be included in the analysis as a 36 weeks PMA response. The number of responses falling outside the scheduled 7-day window for each timepoint will be reported. The number of responses derived from infant medical notes will be reported.

If the participant has reported at an earlier time point that they are no longer lactating (on an expressing log) or no longer human milk feeding (by SMS), then later SMS timepoints will also be defined as no exclusive human milk feeding.

***Secondary outcome: Proportion with any human milk feeding***

This outcome is assessed at 36 weeks' PMA with the same process as above. It is defined as any human milk intake in the reference period (the 24 hours prior to the question being asked). If a participant has two babies, the outcome will be classified with reference to both infants (any human milk intake by either infant).

If the participant has reported at an earlier time point that they are no longer lactating (on an expressing log) or no longer human milk feeding (by SMS), then later SMS timepoints will also be defined as no human milk feeding.

***Secondary outcome: Average maternal anxiety score***

Maternal anxiety will be measured by the STAI-6, at baseline and day 21. The outcome is formed by the day 21 average score, adjusted for baseline.

***Secondary outcome: Average maternal distress score***

Maternal stress reaction to the trauma of preterm birth will be measured by the PCL-5 at day 21.

***Process indicator: Time spent in skin-to-skin contact with infant***

This indicator is measured by questionnaire on day 21. It is measured in number of hours of skin to skin contact by the participant with any of her infants (that is, the sum of the hours spent in skin to skin contact by the participant with both infants if there are two).

***Process indicator: Number of expressing episodes***

This indicator is measured via the expressing log on day 21. It is the number of episodes of expressing recorded by the participant in a 24-hour period. The 24-hour period is defined as per the primary outcome. Expressing two breasts simultaneously is recorded on the log as one expressing episode.

As noted above, the way in which participants record sessions where they have used more than one container for each breast will affect the calculation of an accurate number of expressing episodes. Therefore, the following exclusions apply for this outcome:

- Where an expressing session has been recorded with duration of zero minutes, it will be excluded
- Where the start time and duration of expressing sessions are identical, duplicate sessions will be excluded
- Where the start time of an expressing session is within the duration of a prior session (i.e. the session timings overlap), the later session will be excluded
- Where the start time of an expressing session is less than 10 minutes after a prior session is completed, the later session will be excluded

***Process indicator: Time spent expressing***

This indicator is measured via the expressing log on day 21. It is the sum of the number of minutes recorded for each expressing session in a 24-hour period. The 24-hour period is defined as per the primary outcome. The following exclusion applies to this outcome:

- Where the start time and duration of expressing sessions are identical, duplicate time will be excluded

***Process indicators without statistical inference***

In addition to the process indicators listed individually above, several other process indicators will be reported with summary statistics and without statistical inferences. These are:

- Perception of low milk supply on day 21 (defined by response to the question “How do you feel about your milk supply today?” of “I think I don’t have enough milk”)
- Number of direct breastfeeds on day 21 (defined by response to the question “Thinking of the 24-hour period starting at 0800 yesterday, how many direct breastfeeds did your baby have, if any? Define a breastfeed as an episode at the breast where you felt the baby was sucking and swallowing some milk. If the baby is offered both breasts at a single episode this is one breastfeed. If the baby stops sucking or swallowing for 30 minutes or more, then any further feeding is a new breastfeed. Please add up all the episodes if you have two babies.”)
- Time spent in skin to skin contact in 24 hours on day 14 (defined as above)

- Expressing episodes in 24 hours on day 14 (defined as above)
- Time spent expressing in 24 hours on day 14 (defined as above)
- Perception of low milk supply on day 14 (defined as above)
- Number of direct breastfeeds on day 14 (defined as above)

### **Secondary analyses**

Analyses will be adjusted for stratification factors used at randomisation. In addition, any secondary outcomes using the highest 24-hour milk weight will be adjusted for the day associated with the highest 24-hour milk weight. Analysis of STAI-6 and PCL-5 scores will use total score. Number/proportion of participants with STAI-6 score >40 and PCL-5 score >33 will also be reported with no statistical testing.

No statistical inferences will be made for the additional process indicators listed above.

### **Pre-specified subgroup analyses**

Pre-specified subgroup analyses will be performed for the primary outcome and two secondary outcomes: exclusive HMF at 36 weeks' PMA; and any HMF at 36 weeks' PMA as follows:-

- According to the stratified groupings for gestational age (23 to 27 weeks' PMA versus 28 to 31 weeks' PMA). Statistical testing using an interaction test will be used for the primary outcome only, reporting the adjusted effect estimate and 95% confidence intervals. This is important as it is generally reported that more mothers of extremely premature babies have worse expressing and HMF outcomes than mothers of very premature babies, and mothers of extremely premature babies may be more anxious and distressed due to the higher risk of morbidity and mortality as gestation decreases. The pre-specified hypothesis is that mother of more preterm babies will see a greater effect of the recording intervention due to their increased level of anxiety. However the reverse effect would not be unexpected as the physiological challenges faced by mothers of the more preterm babies (due to the very early

stage of pregnancy related changes in breast tissue and lactation hormones) may be less amenable to relaxation as an intervention.

- According to the stratified grouping of number of babies alive at time of randomisation (one versus two), with no statistical testing. This is important as mothers of multiples are likely to target higher milk yield and need to establish a higher milk supply to be able to exclusively breastfeed two babies, but may have lower intention to human milk feed and have less time to listen to the recording intervention.
- According to intention to exclusively HMF at discharge, reported by participants at baseline (intention for exclusive HMF versus no intention or unsure), with no statistical testing. This is important as participants with no intention to exclusively breastfeed may make an informed choice to use infant formula even though they have established a good milk supply, and/or may make an informed choice to express more infrequently.

Within the recording intervention group only, the primary outcome will be presented for participants with high and low adherence with the intervention. High adherence is defined as listening to the recording two or more times a day on any non-missing timepoint (day 4, 14 or 21). Low adherence is defined as listening less than twice a day on all non-missing timepoints (day 4, 14 and 21). If this definition is impractical, median values will be used instead. This will be presented as summary statistics, with no statistical inference. Baseline characteristics associated with adherence will also be presented.

Within the recording intervention group only, the primary outcome will be presented for participants reporting that the recording made them feel relaxed at any non-missing timepoint (day 4, 14 or 21) compared with those who felt no change or less relaxation on all non-missing timepoints (day 4, 14 and 21). If this definition is impractical, median values will be used instead. This will be presented as summary statistics, with no statistical inference. Baseline characteristics associated with perception of the intervention will also be presented.

## **Sensitivity analyses**

Statistical inferences will be used for the following sensitivity analyses:-

To assess for potential bias due to the distribution of the timepoints of logs used for the primary outcome by group, the primary outcome analysis will be repeated using only day 21 data. For example, there could be increased loss to follow up in one group and therefore increased use of earlier timepoint logs as the primary outcome which are likely to be lower weight and could therefore bias the primary outcome.

To assess for potential bias due to treatment dilution effect, the primary outcome analysis will be repeated without day 4 logs. This is because participants start the recording intervention between day 0 and day 3 after birth and it is therefore unlikely that a difference in milk yield would be seen as early as day 4.

To assess for potential bias due to incomplete logs, the primary outcome analysis will be repeated excluding any logs reporting less than four expressing sessions.

To assess for potential bias due to the timing of expressing logs, the primary outcome analysis will be repeated excluding any logs submitted outside the scheduled 48 hour window.

Sensitivity analysis relating to missing data is discussed below.

## **Comparison of eligible and recruited population**

Demographic characteristics of the pooled trial population will be compared with aggregate data from the potentially eligible population without statistical inferences, using local routinely collected clinical data (Badgernet, Clevermed). The potentially eligible population is defined as:

- Born between 23+0 and 31+6 weeks of gestation
- Baby is born at one of the recruiting units or transferred into a recruiting unit on day 0, 1 or 2 of life
- Baby is a singleton birth or one of twins

The following demographic characteristics will be described for the firstborn infant (representing the mother – because data is entered into the clinical database using the infant as the unit of measurement whereas the trial recruits mothers as individuals):

- Gestational age at birth
- Parity (primiparous or multiparous)
- Multiple pregnancy
- Mode of delivery
- Mother's age

#### **Significance levels and adjustment of p-values for multiplicity**

95% confidence intervals will be used for all pre-specified outcome comparisons.

Acknowledging the issue of multiple testing and the small size of the study, we have limited the number of trial outcomes tested with statistical inferences to 11 in total. There will be no formal adjustment for multiplicity.

#### **Missing data**

Missing data includes variables that were not provided by participants and erroneous milk volumes that have been excluded (less than zero, or where no empty container weight has been recorded). The analysis will be performed on a complete case basis initially (i.e. only variables that have been provided will be analysed).

Given the reliance on participant-entered data for all outcomes and a significant likelihood of data being missing not at random, missing data may have a significant effect on conclusions. Sensitivity analysis will be performed to explore the pattern of missingness for the primary outcome, using multiple imputation and/or a pattern mixture model as appropriate. Statistical inferences will be made to assess the impact of missing data.

All missing data will be reported and described for each outcome and baseline characteristic. Baseline characteristics may be compared for those with missing expressing logs at day 4, 14 and/or 21 but with

a primary outcome assessment, and those with complete expressing logs. These will be presented using summary statistics.

Data submitted before a change of consent/withdrawal will be used for analysis, unless the withdrawal is due suspicion of fraudulent data. This is in line with the stated reason for data processing during the consent process of 'a task in the public interest' (research).

### **Statistical software employed**

Stata/SE will be used for statistical analysis.

### **Safety data analysis**

Safety reporting is not required for this trial due to the nature of the recording intervention. Themes from any negative free text comments will be summarised.

### **Additional exploratory analysis**

Analyses not specified in the analysis protocol will be exploratory in nature and will be documented in a separate statistical analysis plan. Post-hoc analysis requested by the oversight committees, a journal editor or referees will be labelled explicitly as such.



## Appendix E: Risk Assessment for the randomised trial (related to Chapter 6)

| Potential risk                                                                | Mitigation strategy                                                                                                                                                                                                                                                                                                                                                                              | Monitoring (frequency)                                                        | Evidence for mitigation                                                                                                                                             |
|-------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Using the intervention recording could harm the participant</b>            | Parental input into recording modification. Instructions provided to first listen in a private place, as suggested by parents                                                                                                                                                                                                                                                                    | Free text comments; participant report recording perception (3 monthly)       | Partial mitigation. Some evidence of minor harm – dislike of recording, feeling it made participants less relaxed, comments that it was boring/ a chore             |
| <b>Inadequate recruitment</b>                                                 | Active promotion of the trial. Bringing more sites online. Site staff training                                                                                                                                                                                                                                                                                                                   | Recruitment numbers (monthly)                                                 | Mitigated – recruitment target achieved                                                                                                                             |
| <b>Imbalanced allocation</b>                                                  | An independent statistician will validate the randomisation schedule                                                                                                                                                                                                                                                                                                                             | Monitoring report (twice only)                                                | Mitigated – balanced allocation                                                                                                                                     |
| <b>Allocation could be predicted</b>                                          | Stratified block randomisation algorithm using a probabilistic mechanism, prepared by independent statistician                                                                                                                                                                                                                                                                                   | Monitoring report (twice only)                                                | Mitigated – allocation could not be predicted                                                                                                                       |
| <b>Low adherence with intervention</b>                                        | Covered in PIS and consent training (description of intervention). Check-in contact on day 4-7 allows participants to ask questions/acts as a reminder. Participants in the intervention group sent text message on day 9 and 17 to remind them to keep listening to the recording                                                                                                               | Free text comments; participant report of listening frequency (three monthly) | Partial mitigation. High adherence in those who provided data                                                                                                       |
| <b>Contamination of control group</b>                                         | Covered in PIS and consent training (emphasise equipoise). No equivalent recording is available to participants – other soundtracks are generally for term infants/breastfeeding or have an off-putting voice/length/structure for NICU parents                                                                                                                                                  | Participant report of use of other relaxation (three monthly)                 | Partial mitigation. Some level of relaxation use in control group but unknown if this was because of contamination or background rate                               |
| <b>Disengagement/ loss to follow up (potentially unbalanced between arms)</b> | Covered in PIS and consent training (understanding nature of the trial). Automated reminders before and after logging days. Long term endpoints are simple text message responses so minimal effort required from participant. Preparatory email sent to participants before each SMS. Automated repeat SMS if not completed. Follow up by email for non-responders to SMS. Sensitivity analyses | Data completeness                                                             | Partial mitigation. Some unbalanced loss to follow up. Sensitivity analyses did not suggest this influenced conclusions                                             |
| <b>Inaccurate data (participants weighing and recording)</b>                  | Site staff training on weighing procedure and how to train participants. Competency of participant with weighing will be recorded at randomisation and again at day 4-7 check-in contact. Extra supporting material is provided, both written (info sheet and sticker on                                                                                                                         | Blinded data downloads (three monthly)                                        | Partial mitigation. Some erroneous values were recorded. Some issues with ways of recording single bottles/use of multiple bottles were identified during the trial |

|                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                 |                                                                                                                                                              |
|-----------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                                               | weighing scale) and audio visual. Contact details are provided on weighing scales, cool bag and pens in case participants need to ask for help                                                                                                                                                                                                                                                                                                           |                                                                 | and then accounted for in analysis                                                                                                                           |
| <b>Paper CRFs entered by CI could be influenced by bias due to involvement in recruitment</b> | Participants are encouraged to enter data into eCRF. Double entry of paper CRFs if CI involved.                                                                                                                                                                                                                                                                                                                                                          | Programme team audit of paper CRFs before data lock (once only) | Mitigated – no inaccuracies found on audit                                                                                                                   |
| <b>Participant difficulty with electronic CRF process</b>                                     | Resources created on how to use electronic forms with key difficulties highlighted – guidance sheet for site staff, participant leaflet and participant video on website. Site staff training and support offered to participants                                                                                                                                                                                                                        | Rate of paper CRF return (informal)                             | Partial mitigation. Most CRFs filled in electronically                                                                                                       |
| <b>Delayed data entry and potential loss in the post when paper CRFs used</b>                 | Sites encouraged to scan paper forms before postage if possible. LimeSurvey upload option available for participants to upload a photograph of paper forms rather than post                                                                                                                                                                                                                                                                              | Rate of paper CRF return and LimeSurvey use (informal)          | Partial mitigation. Minimal return of paper CRFs once LimeSurvey photos an option                                                                            |
| <b>Change of contact details before final follow up</b>                                       | Mobile phone number and email address will both be requested.                                                                                                                                                                                                                                                                                                                                                                                            | Data completeness. Error messages when SMS sent (monthly)       | Partial mitigation. A small number of mobile phone numbers returned error message but alternative contact details were available                             |
| <b>IT failure, including failure of automated reminders</b>                                   | Validation procedures prior to trial opening                                                                                                                                                                                                                                                                                                                                                                                                             | Error messages/unsent notifications (monthly)                   | Partial mitigation. There were some anomalies of automated messages but these were identified and participants contacted to apologise/send appropriate links |
| <b>Risk of requesting follow up information in cases where an infant may have died</b>        | Parents have been involved in wording of CRFs for sensitivity. Site staff encouraged to access bereavement training to improve their communication with participants undergoing bereavement. At surgical sites it is unlikely that death would take place at another hospital. For non-surgical sites, site staff will manually check for deaths elsewhere at 32 weeks' PMA. Withdrawal form automatically prevents further notifications being sent out | Discussion with site staff (informal)                           | Partial mitigation. No known cases of bereaved parents being asked for data entry                                                                            |
| <b>Errors in deriving the primary, major secondary or complex outcomes during programming</b> | Second statistician will perform a validation analysis of the primary outcome                                                                                                                                                                                                                                                                                                                                                                            | Validation process (once only)                                  | Mitigation. No errors identified by independent validation                                                                                                   |
| <b>CI involvement in recruitment as well as analysis - could</b>                              | Programs will be checked by two statisticians after creation by CI. No access to unblinded data for the CI until                                                                                                                                                                                                                                                                                                                                         | Checking process (once only)                                    | Partial mitigation. Programs created using blinded data downloads.                                                                                           |

|                                                                                                      |                                                                                                                                                            |                                       |                                                                                                                                                       |
|------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>compromise the independence of the statistical analysis</b>                                       | data lock. Interim analysis and monitoring checks to be run by an independent statistician or the programming team.                                        |                                       | No major issues identified on program checks. DMC unblinded report and three monthly monitoring prepared by independent statistician/programming team |
| <b>Transmission of infectious disease between participants and research staff (pandemic setting)</b> | Procedures designed with non-contact back up options e.g. electronic consent, pre-quarantine of weighing scale and other equipment to give to participants | Discussion with site staff (informal) | Mitigation. No known transmission of infection                                                                                                        |

## Appendix F: Tables relating to the randomised trial (Chapter 6)

**Table F.5: Sensitivity analyses (primary outcome)**

|                                                                                                                   | Relaxation<br>Group (n = 68) | Control<br>Group (n = 64) | Unadjusted MD<br>(95% CI) | Adjusted MD<br>(95% CI)   | p<br>value <sup>46</sup> |
|-------------------------------------------------------------------------------------------------------------------|------------------------------|---------------------------|---------------------------|---------------------------|--------------------------|
| <b>Primary Outcome (PO): Highest 24-hour milk weight on day 4, 14 or 21 (grams)</b>                               |                              |                           |                           |                           |                          |
| Mean (SD)                                                                                                         | 596.7<br>(433.6)             | 467.7<br>(350.2)          | 129.1<br>(-20.8 to 278.9) | 73.9<br>(-61.7 to 209.5)  | 0.28                     |
| Median [IQR]                                                                                                      | 521.4<br>[254.6 to 902.7]    | 397.8<br>[176.6 to 719.2] |                           |                           |                          |
| Min to max                                                                                                        | (2 to 1926)                  | (7 to 1274)               |                           |                           |                          |
| Missing                                                                                                           | 16                           | 8                         |                           |                           |                          |
| <b>Sensitivity analysis 1: Using only day 21 milk weight (grams)</b>                                              |                              |                           |                           |                           |                          |
| Mean (SD)                                                                                                         | 596.4<br>(434.8)             | 521.1<br>(325.9)          | 75.3<br>(-86.9 to 237.5)  | 73.0<br>(-91.9 to 237.9)  | 0.38                     |
| Median [IQR]                                                                                                      | 547<br>[247.5 to 938.4]      | 473.8<br>[243 to 735.3]   |                           |                           |                          |
| Min to max                                                                                                        | (0 to 1926)                  | (65 to 1274)              |                           |                           |                          |
| Excluded versus PO                                                                                                | 5                            | 13                        |                           |                           |                          |
| <b>Sensitivity Analysis 2: Using multiple imputation for all missing 24-hour weights (grams)</b>                  |                              |                           |                           |                           |                          |
| Mean (SD)                                                                                                         | 612.1<br>(433.7)             | 533.9<br>(369.2)          | 78.2<br>(-63.5 to 219.9)  | 71.3<br>(-64.3 to 207.0)  | 0.30                     |
| Added versus PO                                                                                                   | 16                           | 8                         |                           |                           |                          |
| <b>Sensitivity analysis 3: Excluding day 4 milk weight (grams)</b>                                                |                              |                           |                           |                           |                          |
| Mean (SD)                                                                                                         | 603.2<br>(437.2)             | 529.4<br>(331.7)          | 73.8<br>(-83.3 to 231.0)  | 46.3<br>(-108.9 to 201.5) | 0.56                     |
| Median [IQR]                                                                                                      | 521.4<br>[247.5 to 938.4]    | 483.2<br>[269.8 to 783.3] |                           |                           |                          |
| Min to max                                                                                                        | (0 to 1926)                  | (30 to 1274)              |                           |                           |                          |
| Excluded versus PO                                                                                                | 2                            | 9                         |                           |                           |                          |
| <b>Sensitivity analysis 4: Excluding all expressing logs with less than four sessions logged (grams)</b>          |                              |                           |                           |                           |                          |
| Mean (SD)                                                                                                         | 666.3<br>(420.8)             | 510.1<br>(340.0)          | 156.3<br>(-2.9 to 315.5)  | 108.4<br>(-45.9 to 262.8) | 0.17                     |
| Median [IQR]                                                                                                      | 684.3<br>[336.2 to 945.7]    | 482.2<br>[236 to 745]     |                           |                           |                          |
| Min to max                                                                                                        | (54 to 1926)                 | (60 to 1274)              |                           |                           |                          |
| Excluded versus PO                                                                                                | 7                            | 10                        |                           |                           |                          |
| <b>Sensitivity analysis 5: Excluding expressing logs submitted &gt;48 hours after scheduled timepoint (grams)</b> |                              |                           |                           |                           |                          |
| Mean (SD)                                                                                                         | 596.7<br>(433.6)             | 465.7<br>(350.0)          | 131.0<br>(-18.9 to 280.8) | 72.9<br>(-64.3 to 210.1)  | 0.29                     |
| Median [IQR]                                                                                                      | 521.4<br>[254.6 to 902.7]    | 397.8<br>[176.6 to 719.2] |                           |                           |                          |
| Min to max                                                                                                        | (0 to 1926)                  | (7 to 1274)               |                           |                           |                          |
| Excluded versus PO                                                                                                | 0                            | 0                         |                           |                           |                          |

MD = mean difference. CI = confidence interval; SD = standard deviation; IQR = interquartile range.  
Missing observations are excluded from percentage denominators

<sup>46</sup>Adjusted analysis

**Table F.6: Measures of data quality**

|                                                                                          | Relaxation Group<br>(n = 68) | Control Group<br>(n = 64) |
|------------------------------------------------------------------------------------------|------------------------------|---------------------------|
| <b>Logs submitted inside the scheduled window<sup>47</sup>; n (% of submitted logs):</b> |                              |                           |
| Day 4                                                                                    | 50/51 (98)                   | 52/53 (98)                |
| Day 14                                                                                   | 46/46 (100)                  | 45/45 (100)               |
| Day 21                                                                                   | 48/48 (100)                  | 43/43 (100)               |
| <b>Logs with erroneous data removed; n (% of submitted logs):</b>                        |                              |                           |
| Day 4                                                                                    | 3/51 (5.9)                   | 5/53 (9.4)                |
| Day 14                                                                                   | 1/46 (2.2)                   | 3/45 (6.7)                |
| Day 21                                                                                   | 4/48 (8.3)                   | 2/43 (4.7)                |
| <b>Logs with fewer than four expressing sessions; n (% of submitted logs):</b>           |                              |                           |
| Day 4                                                                                    | 10/51 (19.6)                 | 13/53 (24.5)              |
| Day 14                                                                                   | 7/46 (15.2)                  | 3/45 (6.7)                |
| Day 21                                                                                   | 6/48 (12.5)                  | 4/43 (9.3)                |
| <b>Day 4 as the timepoint contributing to primary outcome = N</b>                        |                              |                           |
| Log available, n/N (%):                                                                  |                              |                           |
| Day 14                                                                                   | 3/8 (37.5)                   | 5/14 (35.7)               |
| Day 21                                                                                   | 6/8 (75.0)                   | 4/14 (28.6)               |
| <b>Day 14 as the timepoint contributing to primary outcome = N</b>                       |                              |                           |
| Log available, n/N (%):                                                                  |                              |                           |
| Day 4                                                                                    | 9/11 (81.8)                  | 14/15 (93.3)              |
| Day 21                                                                                   | 8/11 (72.7)                  | 12/15 (80.0)              |

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<sup>47</sup>The scheduled window is 48 hours – for example a day 4 log that is started on day 4, day 5 or before 10am on day 6 is inside the schedule window

**Table F.7: Baseline characteristics according to intervention adherence (relaxation group only)**

|                                                                                    | Higher adherence<br>(n = 21) | Lower adherence<br>(n = 31) |
|------------------------------------------------------------------------------------|------------------------------|-----------------------------|
| <b>Mother's ethnicity, n (%)</b>                                                   |                              |                             |
| White                                                                              | 14 (66.7)                    | 18 (58.1)                   |
| Asian or Asian British                                                             | 4 (19.0)                     | 3 (9.7)                     |
| Black, African, Black British or Caribbean                                         | 2 (9.5)                      | 8 (25.8)                    |
| Other                                                                              | 1 (4.8)                      | 1 (3)                       |
| Prefer not to say                                                                  | 0                            | 1 (3.2)                     |
| <b>Mother's age (years), mean (SD)</b>                                             | 33.1 (5.9)                   | 33.0 (6.7)                  |
| <b>Index of Multiple Deprivation quintile<sup>48</sup>, n (%)</b>                  |                              |                             |
| 1 (Most deprived)                                                                  | 4 (19.0)                     | 8 (25.8)                    |
| 2                                                                                  | 4 (19.0)                     | 3 (9.7)                     |
| 3                                                                                  | 3 (14.3)                     | 5 (16.1)                    |
| 4                                                                                  | 6 (28.6)                     | 3 (9.7)                     |
| 5 (Least deprived)                                                                 | 4 (19.0)                     | 12 (38.7)                   |
| <b>Age at leaving full-time education, n (%)</b>                                   |                              |                             |
| 16 years old or less                                                               | 1 (4.8)                      | 2 (6.5)                     |
| 17 or 18 years old                                                                 | 6 (28.6)                     | 4 (12.9)                    |
| 19 years old or more                                                               | 12 (57.1)                    | 22 (71.0)                   |
| Still in full time education                                                       | 2 (9.5)                      | 2 (6.5)                     |
| Prefer not to say                                                                  | 0                            | 1 (3.2)                     |
| <b>Lives with a partner, n (%)</b>                                                 | 19 (90.5)                    | 29 (93.5)                   |
| <b>Current smoker, n (%)</b>                                                       | 0                            | 4 (12.9)                    |
| <b>Time from birth to first expression of milk (hours), median (IQR)</b>           | 5 (3 – 12)                   | 6 (2.8 – 15)                |
| <b>Intention for exclusive breastmilk<sup>49</sup> at time of discharge, n (%)</b> | 14 (66.7)                    | 22 (71.0)                   |
| <b>STAI<sup>50</sup> score at randomisation (score range 20 - 80), mean (SD)</b>   | 55.2 (13.5)                  | 53.4 (14.9)                 |
| Score >40                                                                          | 16 (80.0)                    | 24 (82.8)                   |
| Missing                                                                            | 1                            | 2                           |
| <b>Mode of delivery<sup>51</sup>, n (%)</b>                                        |                              |                             |
| Vaginal birth                                                                      | 5 (23.8)                     | 10 (32.3)                   |
| Caesarean birth                                                                    | 16 (76.2)                    | 21 (67.8)                   |
| <b>Multiple pregnancy, n (%)</b>                                                   | 2 (9.5)                      | 6 (19.4)                    |
| <b>Primiparous, n (%)</b>                                                          | 10 (47.6)                    | 19 (61.3)                   |
| <b>Recruiting centre, n (%)</b>                                                    |                              |                             |
| 1                                                                                  | 12 (57.1)                    | 19 (61.3)                   |
| 2                                                                                  | 3 (14.3)                     | 9 (29.0)                    |
| 3                                                                                  | 2 (9.5)                      | 2 (6.5)                     |
| 4                                                                                  | 4 (19.0)                     | 1 (3.2)                     |

<sup>48</sup>Index of multiple deprivation: assigned nationally by postcode to create comparable classification areas of similar deprivation level across the country

<sup>49</sup>Exclusive breastfeeding defined as “your baby would be drinking only your breastmilk and no infant formula”

<sup>50</sup>Six item STAI, transformed to an equivalent score to 20 item STAI

<sup>51</sup>If two infants, the most invasive method of birth is reported, where caesarean birth is more invasive than instrumental birth and instrumental birth is more invasive than vaginal birth

|                                                                 | Higher adherence<br>(n = 21) | Lower adherence<br>(n = 31) |
|-----------------------------------------------------------------|------------------------------|-----------------------------|
| <b>Gestational age at birth (weeks), n (%)</b>                  |                              |                             |
| Median [IQR]                                                    | 28.7 (27.1 – 30.6)           | 27.0 (25.4 – 29.0)          |
| 23 to < 26 weeks                                                | 5 (23.8)                     | 9 (29.0)                    |
| 26 to < 28 weeks                                                | 2 (9.5)                      | 12 (38.7)                   |
| 28 to < 30 weeks                                                | 6 (28.6)                     | 6 (19.4)                    |
| 30 to < 32 weeks                                                | 8 (38.1)                     | 4 (12.9)                    |
| <b>Ventilated at randomisation (one or both infants), n (%)</b> | 10 (47.6)                    | 7 (22.6)                    |

**Table F.8: Baseline characteristics according to primary outcome missing status**

|                                                                                  | Primary Outcome available (n = 108) | Primary Outcome missing (n = 24) |
|----------------------------------------------------------------------------------|-------------------------------------|----------------------------------|
| <b>Mother's ethnicity, n (%)</b>                                                 |                                     |                                  |
| White                                                                            | 62 (57.9)                           | 10 (71.4)                        |
| Asian or Asian British                                                           | 19 (17.8)                           | 3 (21.4)                         |
| Black, African, Black British or Caribbean                                       | 20 (18.7)                           | 1 (7.1)                          |
| Mixed or multiple ethnic groups                                                  | 3 (2.8)                             | 0                                |
| Other                                                                            | 2 (1.9)                             | 0                                |
| Prefer not to say                                                                | 1 (0.9)                             | 0                                |
| Missing                                                                          | 1                                   | 10                               |
| <b>Mother's age (years), mean (SD)</b>                                           | 33.0 (6.0)                          | 32.0 (7.7)                       |
| Median [IQR]                                                                     | 33 [30 to 36]                       | 32 [26 to 35]                    |
| (Min to max)                                                                     | (21 to 55)                          | (19 to 50)                       |
| <b>Index of Multiple Deprivation quintile<sup>52</sup>, n (%)</b>                |                                     |                                  |
| 1 (Most deprived)                                                                | 19 (17.6)                           | 5 (21.7)                         |
| 2                                                                                | 23 (21.3)                           | 3 (13.0)                         |
| 3                                                                                | 18 (16.7)                           | 4 (17.4)                         |
| 4                                                                                | 22 (20.4)                           | 9 (39.1)                         |
| 5 (Least deprived)                                                               | 26 (24.1)                           | 2 (8.7)                          |
| Missing                                                                          | 0                                   | 1                                |
| <b>Age at leaving full-time education, n (%)</b>                                 |                                     |                                  |
| 16 years old or less                                                             | 11 (10.3)                           | 3 (21.4)                         |
| 17 or 18 years old                                                               | 21 (19.6)                           | 3 (21.4)                         |
| 19 years old or more                                                             | 69 (64.5)                           | 8 (57.1)                         |
| Still in full time education                                                     | 5 (4.7)                             | 0                                |
| Prefer not to say                                                                | 1 (0.9)                             | 0                                |
| Missing                                                                          | 1                                   | 10                               |
| <b>Lives with a partner, n (%)</b>                                               | 96 (89.7)                           | 12 (85.7)                        |
| Missing                                                                          | 1                                   | 10                               |
| <b>Current smoker, n (%)</b>                                                     | 7 (6.5)                             | 1 (7.1)                          |
| Missing                                                                          | 1                                   | 10                               |
| <b>Time from birth to first expression of milk (hours), median (IQR)</b>         | 6 [3 to 12]                         | 7 [2 to 12]                      |
| (Min to max)                                                                     | (0 to 96)                           | (1 to 72)                        |
| Missing                                                                          | 2                                   | 10                               |
| <b>Intention for exclusive breastmilk<sup>53</sup>, n (%)</b>                    | 76 (71.0)                           | 9 (64.3)                         |
| Missing                                                                          | 1                                   | 10                               |
| <b>STAI<sup>54</sup> score at randomisation (score range 20 - 80), mean (SD)</b> | 52.1 (14.3)                         | 49.8 (14.2)                      |
| Median [IQR]                                                                     | 52 [40 to 63]                       | 47 [40 to 54]                    |
| (Min to max)                                                                     | (20 to 80)                          | (33 to 77)                       |
| Score >40                                                                        | 73 (74.5)                           | 7 (58.3)                         |
| Missing                                                                          | 10                                  | 12                               |

<sup>52</sup>Index of multiple deprivation: assigned nationally by postcode to create comparable classification areas of similar deprivation level across the country

<sup>53</sup>Exclusive breastfeeding defined as "your baby would be drinking only your breastmilk and no infant formula" at discharge from hospital

<sup>54</sup>Six item STAI, transformed to an equivalent score to 20 item STAI



|                                                                 | Primary Outcome available (n = 108) | Primary Outcome missing (n = 24) |
|-----------------------------------------------------------------|-------------------------------------|----------------------------------|
| <b>Mode of delivery<sup>55</sup>, n (%)</b>                     |                                     |                                  |
| Vaginal birth                                                   | 44 (40.7)                           | 13 (54.2)                        |
| Instrumental birth                                              | 0 (0.0)                             | 0 (0.0)                          |
| Caesarean birth                                                 | 64 (59.3)                           | 11 (45.8)                        |
| <b>Multiple pregnancy, n (%)</b>                                | 16 (14.8)                           | 4 (16.7)                         |
| <b>Primiparous, n (%)</b>                                       | 64 (59.8)                           | 10 (58.8)                        |
| Missing                                                         | 1                                   | 7                                |
| <b>Recruiting centre, n (%)</b>                                 |                                     |                                  |
| 1                                                               | 65 (60.2)                           | 12 (50.0)                        |
| 2                                                               | 25 (23.1)                           | 7 (29.2)                         |
| 3                                                               | 8 (7.4)                             | 2 (8.3)                          |
| 4                                                               | 10 (9.3)                            | 3 (12.5)                         |
| <b>Gestational age at birth (weeks), n (%)</b>                  |                                     |                                  |
| Median [IQR]                                                    | 28 [26 to 30]                       | 28 [26 to 30]                    |
| (Min to Max)                                                    | (23 to 32)                          | (24 to 32)                       |
| 23 to < 26 weeks                                                | 28 (25.9)                           | 6 (25.0)                         |
| 26 to < 28 weeks                                                | 31 (28.7)                           | 6 (25.0)                         |
| 28 to < 30 weeks                                                | 24 (22.2)                           | 5 (20.8)                         |
| 30 to < 32 weeks                                                | 25 (23.1)                           | 7 (29.2)                         |
| <b>Ventilated at randomisation (one or both infants), n (%)</b> | 39 (36.1)                           | 8 (33.3)                         |

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<sup>55</sup>If two infants, the most invasive method of birth is reported, where caesarean birth is more invasive than instrumental birth and instrumental birth is more invasive than vaginal birth

**Table F.9: Baseline characteristics according to presence of complete logs**

|                                                                                    | Three logs available <sup>56</sup> (n = 80) | Less than three logs available (n = 52) |
|------------------------------------------------------------------------------------|---------------------------------------------|-----------------------------------------|
| <b>Mother's ethnicity, n (%)</b>                                                   |                                             |                                         |
| White                                                                              | 48 (60.0)                                   | 24 (58.5)                               |
| Asian or Asian British                                                             | 12 (15.0)                                   | 10 (24.4)                               |
| Black, African, Black British or Caribbean                                         | 16 (20.0)                                   | 5 (12.2)                                |
| Mixed or multiple ethnic groups                                                    | 2 (2.5)                                     | 1 (2.4)                                 |
| Other                                                                              | 1 (1.3)                                     | 1 (2.4)                                 |
| Prefer not to say                                                                  | 1 (1.3)                                     | 0                                       |
| Missing                                                                            | 0                                           | 11                                      |
| <b>Mother's age (years), mean (SD)</b>                                             | 33.3 (4.4)                                  | 32.0 (8.5)                              |
| Median [IQR]                                                                       | 34 [31 to 36]                               | 32 [26 to 35]                           |
| (Min to max)                                                                       | (24 to 44)                                  | (19 to 55)                              |
| <b>Index of Multiple Deprivation quintile<sup>57</sup>, n (%)</b>                  |                                             |                                         |
| 1 (Most deprived)                                                                  | 11 (13.8)                                   | 13 (25.5)                               |
| 2                                                                                  | 15 (18.8)                                   | 11 (21.6)                               |
| 3                                                                                  | 13 (16.3)                                   | 9 (17.6)                                |
| 4                                                                                  | 18 (22.5)                                   | 13 (25.5)                               |
| 5 (Least deprived)                                                                 | 23 (28.8)                                   | 5 (9.8)                                 |
| Missing                                                                            | 0                                           | 1                                       |
| <b>Age at leaving full-time education, n (%)</b>                                   |                                             |                                         |
| 16 years old or less                                                               | 7 (8.8)                                     | 7 (17.1)                                |
| 17 or 18 years old                                                                 | 13 (16.3)                                   | 11 (26.8)                               |
| 19 years old or more                                                               | 56 (70.0)                                   | 21 (51.2)                               |
| Still in full time education                                                       | 3 (3.8)                                     | 2 (4.9)                                 |
| Prefer not to say                                                                  | 1 (1.3)                                     | 0                                       |
| Missing                                                                            | 0                                           | 11                                      |
| <b>Lives with a partner, n (%)</b>                                                 | 74 (92.5)                                   | 34 (82.9)                               |
| Missing                                                                            | 0                                           | 11                                      |
| <b>Current smoker, n (%)</b>                                                       | 5 (6.3)                                     | 3 (7.3)                                 |
| Missing                                                                            | 0                                           | 11                                      |
| <b>Intensive care in first 48 hours after birth, n (%)</b>                         | 1 (1.3)                                     | 0 (0.0)                                 |
| <b>Time from birth to first expression of milk (hours), median (IQR)</b>           | 6 [3 to 12]                                 | 7 [2 to 15]                             |
| (Min to max)                                                                       | (0 to 48)                                   | (1 to 96)                               |
| Missing                                                                            | 1                                           | 11                                      |
| <b>Intention for exclusive breastmilk<sup>58</sup> at time of discharge, n (%)</b> | 57 (71.3)                                   | 28 (68.3)                               |
| Missing                                                                            | 0                                           | 11                                      |

<sup>56</sup>Day 4, 14 and 21<sup>57</sup>Index of multiple deprivation: assigned nationally by postcode to create comparable classification areas of similar deprivation level across the country<sup>58</sup>Exclusive breastfeeding defined as "your baby would be drinking only your breastmilk and no infant formula"

|                                                                                  | Three logs available <sup>56</sup> (n = 80) | Less than three logs available (n = 52) |
|----------------------------------------------------------------------------------|---------------------------------------------|-----------------------------------------|
| <b>STAI<sup>59</sup> score at randomisation (score range 20 - 80), mean (SD)</b> | 51.9 (14.7)                                 | 51.7 (13.4)                             |
| Median [IQR]                                                                     | 52 [40 to 60]                               | 50 [40 to 63]                           |
| (Min to max)                                                                     | (20 to 80)                                  | (20 to 77)                              |
| Score >40                                                                        | 55 (72.4)                                   | 25 (73.5)                               |
| Missing                                                                          | 4                                           | 18                                      |
| <b>Mode of delivery<sup>60</sup>, n (%)</b>                                      |                                             |                                         |
| Vaginal birth                                                                    | 32 (40.0)                                   | 25 (48.1)                               |
| Instrumental birth                                                               | 0 (0.0)                                     | 0 (0.0)                                 |
| Caesarean birth                                                                  | 48 (60.0)                                   | 27 (51.9)                               |
| <b>Multiple pregnancy, n (%)</b>                                                 | 13 (16.3)                                   | 7 (13.5)                                |
| <b>Primiparous, n (%)</b>                                                        | 49 (61.3)                                   | 25 (56.8)                               |
| Missing                                                                          | 0                                           | 8                                       |
| <b>Recruiting centre, n (%)</b>                                                  |                                             |                                         |
| 1                                                                                | 54 (67.5)                                   | 23 (44.2)                               |
| 2                                                                                | 13 (16.3)                                   | 19 (36.5)                               |
| 3                                                                                | 5 (6.3)                                     | 5 (9.6)                                 |
| 4                                                                                | 8 (10.0)                                    | 5 (9.6)                                 |
| <b>Gestational age at birth (weeks), n (%)</b>                                   |                                             |                                         |
| Median [IQR]                                                                     | 28 [26 to 30]                               | 28 [25 to 30]                           |
| (Min to Max)                                                                     | (23 to 32)                                  | (24 to 32)                              |
| 23 to < 26 weeks                                                                 | 19 (23.8)                                   | 15 (28.8)                               |
| 26 to < 28 weeks                                                                 | 23 (28.8)                                   | 14 (26.9)                               |
| 28 to < 30 weeks                                                                 | 20 (25.0)                                   | 9 (17.3)                                |
| 30 to < 32 weeks                                                                 | 18 (22.5)                                   | 14 (26.9)                               |
| <b>Ventilated at randomisation (one or both infants), n (%)</b>                  | 27 (33.8)                                   | 20 (38.5)                               |

<sup>59</sup>Six item STAI, transformed to an equivalent score to 20 item STAI

<sup>60</sup>If two infants, the most invasive method of birth is reported, where caesarean birth is more invasive than instrumental birth and instrumental birth is more invasive than vaginal birth

**Table F.10: Process indicators on day 14 after birth**

|                                                             | Relaxation Group<br>(n = 68) | Control Group<br>(n = 64) |
|-------------------------------------------------------------|------------------------------|---------------------------|
| <b>Skin to skin contact (hours)<sup>61</sup>, mean (SD)</b> | 2.0 (1.7)                    | 1.7 (1.6)                 |
| Median [IQR]                                                | 2 [0 to 3]                   | 2 [0 to 2]                |
| Min to max                                                  | (0 to 6)                     | (0 to 7)                  |
| Missing                                                     | 25                           | 20                        |
| <b>Expressing episodes<sup>62</sup>, mean (SD)</b>          | 5.9 (2.2)                    | 6.0 (1.6)                 |
| Median [IQR]                                                | 6 [5 to 7]                   | 6 [5 to 7]                |
| Min to max                                                  | (0 to 11)                    | (1 to 9)                  |
| Not lactating                                               | 1 (2.0)                      | 0 (0.0)                   |
| Solely breastfeeding                                        | 0 (0.0)                      | 0 (0.0)                   |
| Missing                                                     | 22                           | 19                        |
| <b>Expressing duration (minutes), mean (SD)</b>             | 153.7 (80.6)                 | 169.4 (76.6)              |
| Median [IQR]                                                | 160 [100 to 195]             | 161 [120 to 210]          |
| Min to max                                                  | (0 to 430)                   | (30 to 390)               |
| Missing                                                     | 22                           | 19                        |
| <b>Perception of low milk supply, n (%)</b>                 | 12 (27.9)                    | 19 (43.2)                 |
| Missing                                                     | 25                           | 20                        |
| <b>Direct breastfeeds<sup>63</sup>, mean (SD)</b>           | 0.1 (0.5)                    | 0.0 (0.2)                 |
| Median [IQR]                                                | 0 [0 to 0]                   | 0 [0 to 0]                |
| Min to max                                                  | (0 to 2)                     | (0 to 1)                  |
| Missing                                                     | 41                           | 30                        |

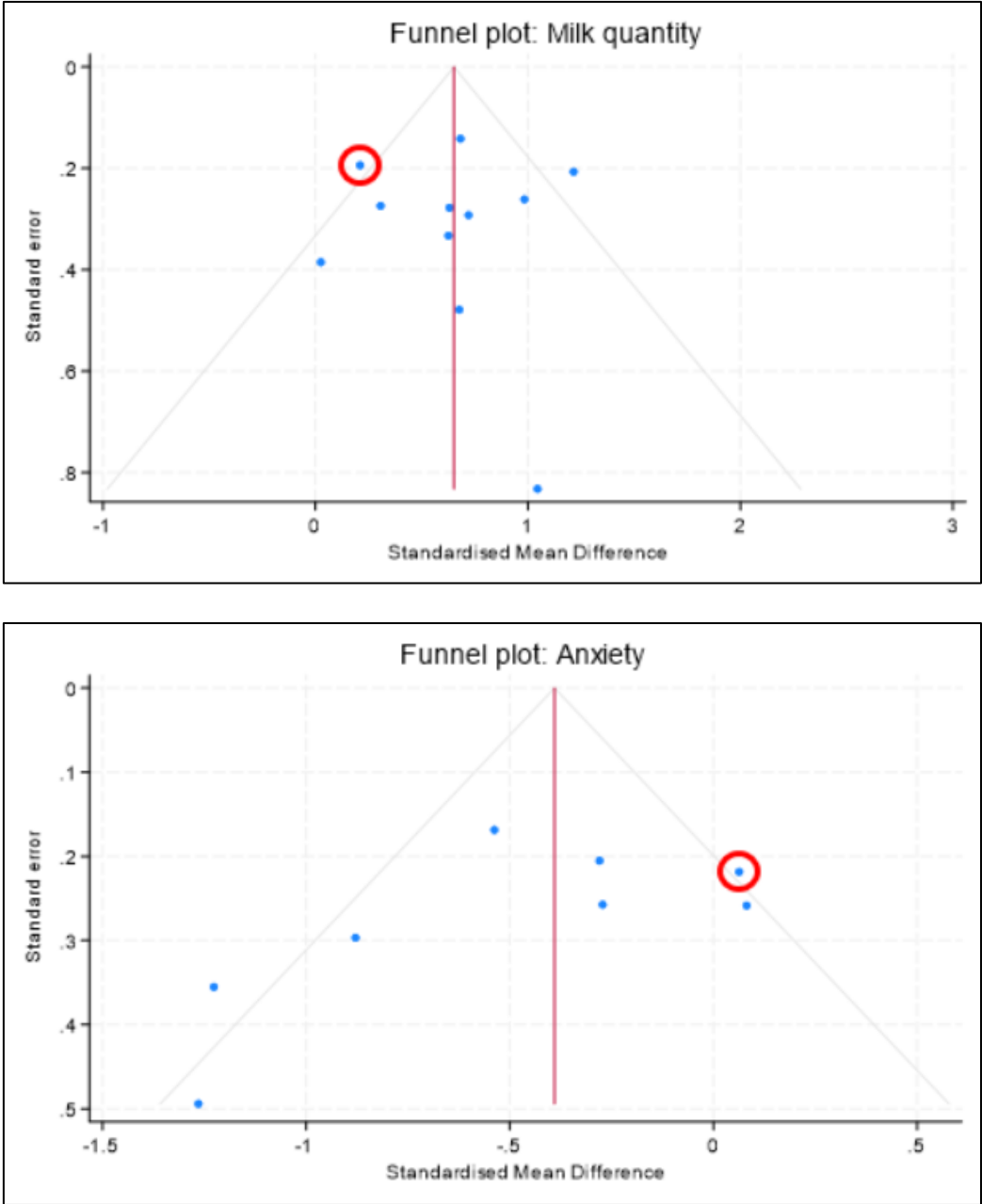
MD = mean difference. Missing data are excluded from percentage denominators

<sup>61</sup>If two infants, then the sum of time spent in skin to skin contact with both

<sup>62</sup>An expressing episode is defined as where two breasts are expressed simultaneously or where two breasts are expressed sequentially, with the start time of the second breast expression within 10 minutes of the end time of the first breast expression or where one breast is expressed alone

<sup>63</sup>A direct breastfeed is defined as an episode at the breast where the mother felt the infant was sucking and swallowing some milk. If the infant is offered both breasts at a single episode this is one breastfeed. If the infant stops sucking or swallowing for 30 minutes or more, then any further feeding is a new breastfeed. If two infants, then the sum of all episodes with both infants

Figure F.2: Funnel plot for meta-analysis with my own trial included (marked in red)



## Appendix G: Dissemination materials related to the randomised trial (Chapter 6)

Easy Read Leaflet (two pages):



### Results Summary

Babies who are born very early (at less than seven months of pregnancy) can't drink milk directly from the breast or a bottle. Because breastmilk is the best milk for babies born very early, parents are asked to 'express' milk from the breasts to give to their babies down a tube into their tummies. We did a research study to see whether listening to a relaxing recording increased the amount of milk people could express, and/or made them less anxious and distressed.

Some people find it hard to express milk however hard they try, and others find it easier. We don't want people to feel judged about the amount of milk they can express or how they feed their babies. You can see more information about breastmilk and breastfeeding on the Bliss website: [www.bliss.org.uk](http://www.bliss.org.uk). To speak to someone about expressing and breastfeeding you can call the **National Breastfeeding Helpline on 0300 100 0212**.

#### What did we do?

132 people were in the trial.

We gave half the people a relaxing recording. We asked them to listen to it while expressing milk.

The other half of the people didn't have the recording.

We asked everyone to tell us about how they expressed milk and asked them to answer questions.

To download the recording you can scan the QR code or go to [www.npeu.ox.ac.uk/express/download-relaxation](http://www.npeu.ox.ac.uk/express/download-relaxation)



#### Did the relaxing recording help people make more milk?

People who had the relaxing recording made a little bit more milk (about 75ml more each day). The trial wasn't big enough to be sure if a difference of this size was because of chance or because of the recording.

A few trials have looked at breastmilk and relaxation. When you put all the results together it looks like relaxation helps people make more milk. Relaxation might have less effect when people have had a baby very early.

There was no difference in how many babies were getting breastmilk later on between the people with and without the relaxing recording.



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REC Reference: 21/LO/0279

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v1.0, 18-Sep-2023

## Did the relaxing recording make people feel better?

There was no difference in how people felt between the two groups

People in the two groups were equally worried.

Three weeks after birth about half of the people in the trial were very worried (enough to be called "clinical anxiety").



People in the two groups were equally distressed.

Three weeks after birth about two in 10 people had a lot of distress (enough to be called "post-traumatic stress").



## How did people feel about the recording?

Most people liked the recording and said it was relaxing.

About one in ten people didn't like the recording.

About one in ten people said they felt less relaxed after listening to the recording.

Some people thought the recording was boring.

Some people would have liked to listen to relaxing music instead.



## Here are some things people said about the recording:

*"When I start listening [to] the recording and meditating before expressing, milk starts falling down my chest from both breasts without me touching at all"*

*"The recording is very monotonous... I feel pressure about listening"*

*"It gives me more energy..."*

*Yes, the recording keeps telling me I can express more for my boys"*

*"It makes me anxious... I didn't find the voice very soothing"*

*"On stressful days it definitely makes me more calm."*

*"Finding time to remember to listen is tricky"*

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*You can find out more detailed results on our website [www.npeu.ox.ac.uk/express](http://www.npeu.ox.ac.uk/express)*

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**EXPRESS Chief Investigator**  
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REC Reference: 21/LO/0279

IRAS ID: 291449

v1.0, 18-Sep-2023



## Results Summary

### What did we do and why?

Babies who are born very early (at less than seven months of pregnancy) can't drink milk directly from the breast or a bottle. Because breastmilk is the best milk for babies born very early, parents are asked to 'express' milk from the breasts to give to their babies down a tube into their tummies.

Many families find that expressing milk for their premature baby can be challenging. The aim of the trial was to find out if listening to a relaxing recording while expressing milk increased the amount people could express and made them feel less anxious or distressed.

The EXPRESS trial included 132 people who gave birth between 23 and 31 weeks of pregnancy (five to seven months).

We gave half the group a relaxing recording to listen to while expressing milk (this was random, like the throw of a dice).

We asked people to tell us about each time they expressed milk on day 4, 14 and 21 after birth and answer some questions. We sent text messages later on to find out how people were feeding their babies.

If you want to download and share the relaxing recording you can find it here (or scan the QR code): [www.npeu.ox.ac.uk/express/download-relaxation](http://www.npeu.ox.ac.uk/express/download-relaxation)



### Did the relaxing recording increase the amount of milk people could express?

The people who had the relaxing recording expressed on average **74 grams** more milk each day than the people who did not. This is roughly the same as 74ml (two and a half ounces).

**We can't say if this difference is the result of chance** or if it is because of the relaxing recording (as the difference is not 'statistically significant'). To be confident that a difference of 74g was because of the recording, we would have needed about 300 people in the trial.

There was no difference between the two groups (the people with the recording and the people without the recording) in how many babies were being fed breastmilk later on (near the due date and four months after the due date).





Another way of being more sure about a trial result is to combine it with other trials, so there are more people overall. When we put the results of all the trials on relaxation and breastmilk together (11 trials including 767 people), it looks like relaxation increases the amount of milk that people make. The average increase over all these studies would be similar to 250ml of milk per day.

These trials used lots of different relaxation techniques in many kinds of families and countries. It's possible that relaxation has a smaller effect when people have had a baby very early, or that some kinds of relaxation work better than others, or that they work differently in people with different experiences.

### Did the relaxing recording make people less anxious or distressed?

There was no evidence that the recording made people less anxious or distressed.

The two groups had the same average scores on the questionnaire when asked about their anxiety. Three weeks after birth, **about half** the people in the trial had a level of anxiety that could be called 'clinical anxiety'.

The two groups had the same average scores on the questionnaire when asked about post-traumatic stress. Three weeks after birth, about **one in five** of the people in the trial had a level of distress that could be called 'post-traumatic stress'.

### Did the relaxing recording change anything else?

There was no difference in the number of times people expressed, how long they expressed or how long they had skin to skin contact with their baby.

### Did people listen to the recording?

Almost everyone said they had listened to the recording at least once (98%).

People said that they listened to the recording three times each day on average.

The people who said that they listened three or more times each day expressed 246g (roughly 246ml) more milk on average than those who said that they listened less than three times each day.

Six out of ten people in the relaxing recording group also used another form of relaxation.

One in four people in the group without the recording used another form of relaxation.

### How did people feel about the recording?

**Three out of five** people liked the recording. **Three out of four** found it relaxing.

About one in ten people disliked the recording and/or said it made them feel less relaxed.

The rest of the people were neutral (they didn't have positive or negative feelings).

A third of people left comments about the recording. The most common positive comments were that people felt relaxed or calmer. The most common negative comments were that listening to the same recording frequently was boring. Several people said they would prefer relaxing music instead of someone talking.

The next page shows some of the comments people made about the recording.

"When I start listening [to] the recording and meditating before expressing, milk starts falling down my chest from both breasts without me touching at all"

"It gives me more energy... Yes, it keeps you wanting to express more... Yes, the recording keeps telling me I can express more for my boys"

"The recording puts me on a more relaxed state of mind that helps with expressing milk and removing so much of the trauma that has occurred in the past few days"

"I believe because my birth and recovery has felt rather traumatic, the recording reminds me to focus on the things that I can do right now and being present while I'm expressing focusing on the love I have for my child as opposed to all the other unsavoury bits..."

### Positive comments

"This morning I really liked the part about putting your other feelings in a book and closing that book. Even though I haven't had skin to skin contact with [baby] this week I like the part that talks about this as my mind goes back to the 3 long cuddles we had..."

"On stressful days it definitely makes me more calm. It's a good length - enough to relax but not too long, once it finishes I pay a bit more attention to expressing & any areas that need a massage etc"

"The study recording has acted as a launchpad to more meditation and as such I have downloaded other apps"

"The first time I listened to it, the parts about imagining your baby nuzzling the breast, or holding your baby, made me feel a bit sad because that's really far off in our situation, but I have got used to it"

"I think I would prefer calming music rather than someone talking"

"If [baby] is having a good day the recording is brilliant I can actually pay attention to it when we have a day which isn't as good I find it hard to concentrate on it, it becomes more of a chore than a relaxation method"

"because of being in hospital there was so much else going on I wasn't able to get the most out of it, even at home my little boy didn't let me listen to it with any kind of commitment"

### Negative or mixed comments

"Finding time to remember to listen is tricky"

"It makes me anxious... I didn't find the voice very soothing, it annoyed me after a time."

"The recording is very monotonous... I feel pressure about listening to the recording, and I don't really like listening to it"

## How did people feel about being in the trial?

Some people said that they enjoyed being part of the trial.



If you have a premature baby and are finding it hard to express milk and breastfeed and want to talk to someone you can call the **National Breastfeeding Helpline** on **0300 100 0212**. They are open from 9:30am to 9:30pm every day. Support is available in English, Welsh, Polish, Bengali and Sylheti.

If you have a premature baby in the neonatal unit, or have had in the past, you can talk to someone about your time in the neonatal unit you can book a video call with a Bliss Champion here: [www.bliss.org.uk/support-via-video-call-form](http://www.bliss.org.uk/support-via-video-call-form) or get support by email: [hello@bliss.org.uk](mailto:hello@bliss.org.uk)

If you are worried about your mental health you can contact your health visitor or your GP.

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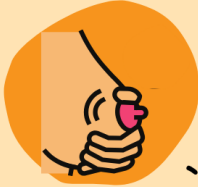
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## Did giving people a relaxation recording help them make more milk after giving birth very early?



### What is the problem?

When people give birth very early (at less than seven months of pregnancy), the baby can't drink milk directly from the breast or a bottle

Because breastmilk helps protect these babies from infection, parents are asked to 'express' milk to give to their babies down a tube into their stomach

It can be hard to express milk, and parents are often worried about their baby and their milk supply

### What did we do?

132 people joined our research study

They had given birth when they were between 5 and 7 months pregnant, in the previous 3 days

We gave half the people a relaxation recording and asked them to listen to it while expressing milk. The other half did not get the recording

You can download the recording here:



**People who got the relaxation recording expressed a little bit more milk (about 75ml more each day)**

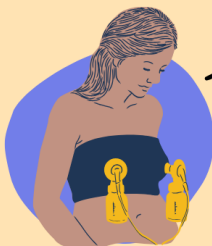
compared to those who didn't have the recording

**The study wasn't big enough to be sure if this was because of chance or because of the recording**

There was no difference in how many babies were getting breastmilk later on

### The relaxation recording had no effect on how anxious or distressed people felt

At three weeks after birth about half the people in the study had 'clinical anxiety' and about one in five people had 'post-traumatic stress'



**Most people liked the recording and felt more relaxed after using it**

About 1 in 10 people didn't like the recording

About 1 in 10 people felt less relaxed after using it

Some people would have liked to listen to relaxing music instead, and to have a variety of relaxing things to listen to

- People who have given birth very early are often anxious and distressed. Expressing milk can be challenging
- Most people enjoyed listening to a relaxing recording
- If the recording had any effect on the amount of milk expressed, this was likely quite small
- Small increases in breastmilk can be important for sick babies

Some people find it hard to express milk however hard they try, and others find it easier. Giving breastmilk to sick babies is one of many things that parents can do to help them, like having skin to skin cuddles, talking to them, changing nappies and more

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For more information about babies born early or sick, see [www.bliss.org.uk](http://www.bliss.org.uk)



## Appendix H: Tables and documents supporting the literature review on expressing pattern and lactation (Chapter 7)

**Table H.11: Exploratory literature review database search terms and flowchart**

**PubMed.** Filters: Human. Final search date: 08/10/2023

|     |                                                                             |
|-----|-----------------------------------------------------------------------------|
| #1. | breastfe* OR (human NEAR milk) OR (breast NEAR milk) OR (mother* NEAR milk) |
| #2. | preterm OR prematur* OR vlbw OR elbw                                        |
| #3. | express* OR pump*                                                           |
| #4. | #1 AND #2 AND #3                                                            |

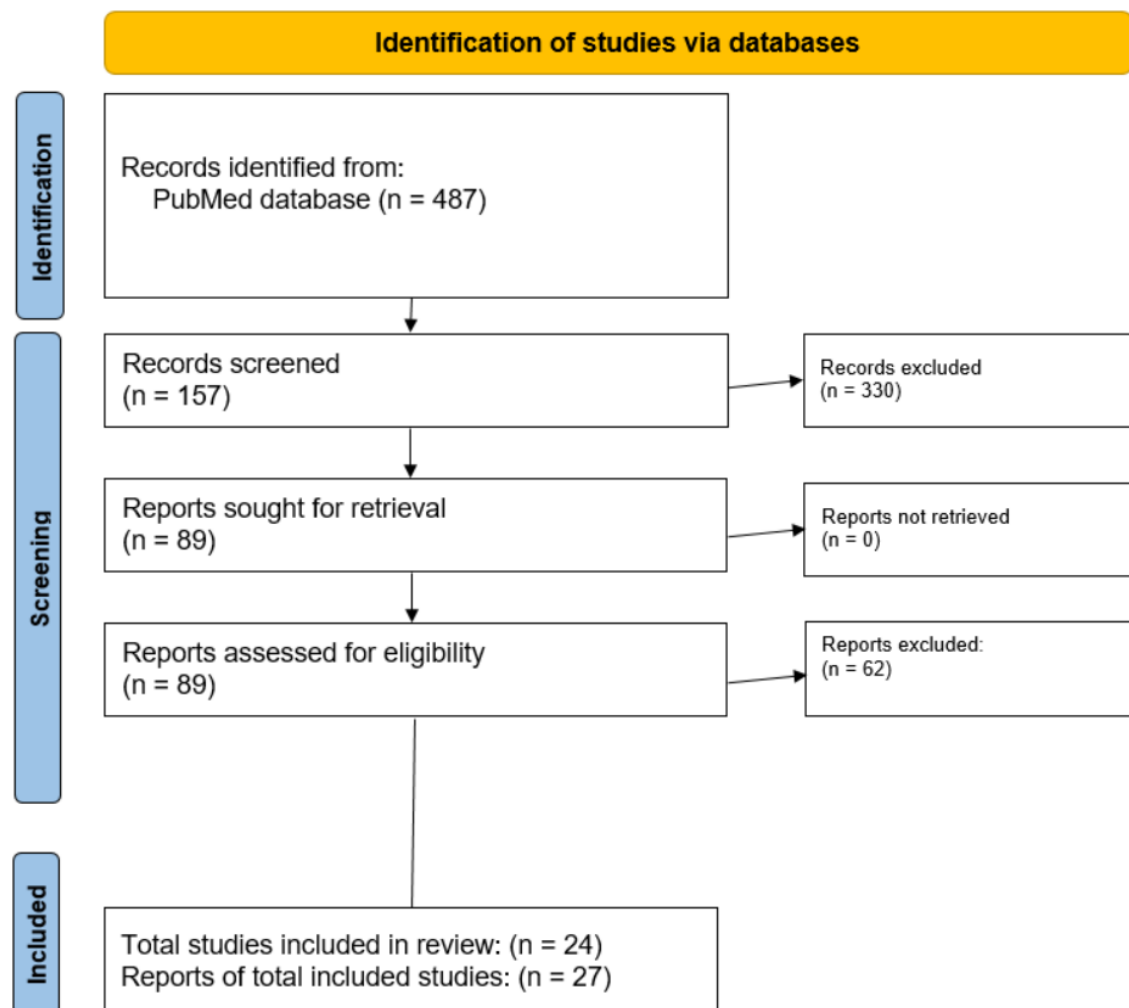


**Table H.12: Exploratory literature search evidence tables**

| Study                                             | Context                                                                  | Study method<br>Confounders                                                                                                                                          | Expressing<br>frequency                                                                     | Outcome                                                               | Effect estimate                                                                                                                                                                                       |
|---------------------------------------------------|--------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Outcome: Any Mother's own breastmilk (MOM)</b> |                                                                          |                                                                                                                                                                      |                                                                                             |                                                                       |                                                                                                                                                                                                       |
| <b>Bin-Nun<br/>2021</b> <sup>276</sup>            | 53 mothers of VLBW infants born ≥24 weeks' PMA; Israel                   | Observational. Adjusted for birth mode, GA, time to 1st expression, prior lactation                                                                                  | Day 21                                                                                      | Any MOM at discharge                                                  | No significant association                                                                                                                                                                            |
| <b>Furman<br/>2002</b> <sup>31</sup>              | 119 mothers of singleton VLBW infants born <33 weeks' PMA and >600g; USA | Observational. Adjusted for age, race, marital status, education, SSC, 1st milk expression, milk yield at baseline, putting the infant to the breast by 35 weeks' CA | ≥5/day at week 3 versus <5/day                                                              | Any MOM at 40 weeks' PMA                                              | 26/30 (86.7%) with MOM at 40wk were in high frequency group. 36/57 (63.2%) without MOM at 40wk were in high frequency group<br><br>Significant in multivariable analysis (no effect estimate, p=0.04) |
|                                                   |                                                                          |                                                                                                                                                                      | ≥5/day at 35 weeks' PMA versus <5/day                                                       |                                                                       | No significant association<br><br>24/30 (80.0%) with MOM at 40wk were in high frequency group. 15/19 (78.9%) without MOM at 40wk were in high frequency group                                         |
| <b>Lau 2007</b> <sup>76</sup>                     | 124 mothers of 'healthy' infants born 26 to 29 weeks' PMA; USA           | Observational. Adjusted for maternal age, BW, milk yield, SSC dose, education, income, parental role alteration score                                                | Two, four, six, eight and ten weeks                                                         | Any MOM at 10wks (or last study visit, which was earliest four weeks) | Univariable significant association (no effect estimate)<br><br>In multivariable analysis, no longer significant                                                                                      |
| <b>Maruyama<br/>2016</b> <sup>136</sup>           | 297 infants born <33 weeks' PMA; Japan                                   | Observational. Adjusted for maternal age, marital status, smoking, parity, time to first expression, GA                                                              | Interval between expressions from birth to discharge from NICU (or cessation of expressing) | Exclusive formula feeding at discharge                                | No significant association<br><br>Adjusted OR 0.85 (95% CI 0.7 - 1.0)                                                                                                                                 |



| Study                                                       | Context                                                                       | Study method<br>Confounders                                                                                                                                           | Expressing<br>frequency                       | Outcome                                                  | Effect estimate                                                                                                                                   |
|-------------------------------------------------------------|-------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|----------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| Patel<br>2019 <sup>153</sup>                                | 415 mothers of infants born <36 weeks’ PMA & VLBW; USA                        | Observational. Adjusted for maternal age, socioeconomic& marital status, ethnicity, education, parity, bf intent, family support, maternal illness, milk yield day 14 | First 14 days after birth                     | Any MOM at discharge                                     | Adjusted OR 7.5 (95% CI 3.3 – 17.1)                                                                                                               |
| Outcome: Exclusive Mother’s own breastmilk (MOM)            |                                                                               |                                                                                                                                                                       |                                               |                                                          |                                                                                                                                                   |
| Gomez-Juge<br>2023 <sup>275</sup>                           | 31 mothers of VPT or VLBW infants in NICU for ≥3 weeks; USA                   | Observational. No adjustment                                                                                                                                          | Cumulative expressing frequency in first week | Exclusive MOM at discharge                               | Median 6.7 times/day (IQR 5.7 to 7.4) for those with exclusive MOM<br>Median 4 times/day (IQR 3.4 to 6.4) for those without exclusive MOM. p=0.03 |
| Ikonen<br>2018 <sup>33</sup>                                | 129 mothers of preterm or LBW infants in NICU at day six after birth; Finland | Observational. No adjustment                                                                                                                                          | Expressing ≥6/day at 2wks of age              | Index of bf status at 2wks (1 = exclusive MOM, 7 = none) | No significant association                                                                                                                        |
| Murase<br>2014 <sup>277</sup>                               | 85 mothers of singleton VPT infants; Japan                                    | Observational. Adjusted for time to first expression & milk yield                                                                                                     | Day one, two and four                         | Any formula feeding at discharge                         | No significant association                                                                                                                        |
|                                                             |                                                                               |                                                                                                                                                                       | Day three ≥6/day, versus <6/day               |                                                          | OR 3.4 (95% CI 1.3 - 8.5)<br>Adjusted OR 1.9 (95% CI 0.6 to 5.4)                                                                                  |
| Outcome: Expressed milk yield within first 28 days of birth |                                                                               |                                                                                                                                                                       |                                               |                                                          |                                                                                                                                                   |
| Bin-Nun<br>2021 <sup>276</sup>                              | 53 mothers of VLBW infants born ≥24 weeks’ PMA; Israel                        | Observational. Adjusted for birth mode, GA, time to 1st expression, prior lactation                                                                                   | Day seven                                     | Milk yield on day 7                                      | No significant association                                                                                                                        |
|                                                             |                                                                               |                                                                                                                                                                       | Day 14                                        | Milk yield on day 14                                     | Significant association (no effect estimate)                                                                                                      |
|                                                             |                                                                               |                                                                                                                                                                       | Day 21                                        | Milk yield on day 21                                     | Significant association (no effect estimate)                                                                                                      |

| Study                                          | Context                                                                       | Study method<br>Confounders                                                                                                                                            | Expressing<br>frequency              | Outcome                                                   | Effect estimate                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|------------------------------------------------|-------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|-----------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>De<br/>Carvalho<br/>1985</b> <sup>272</sup> | 25 mothers<br>of preterm<br>infants<br>admitted to<br>NICU and<br>not bf; USA | RCT with two<br>randomisation<br>opportunities.<br><br>Randomisation<br>for the period of<br>day five to 11. Re-<br>randomisation for<br>the period of day<br>11 to 18 | ≥4/day versus<br><4/day              | Milk yield<br>at day 11<br>or day 18                      | For those who<br>experienced both<br>allocations (in effect,<br>crossover RCT): 342 ± 229<br>mL in high frequency<br>period versus 221 ± 141<br>mL in low frequency<br>period. n = 9. In paired<br>analysis p<0.02<br><br>For those with one<br>allocation only (in effect,<br>parallel arm RCT): 177 ± 96<br>mL in high frequency<br>period versus 123 ± 118<br>mL in low frequency<br>period. n = 7. MD = +54<br>mL (95% CI -104.7 to<br>212.7) |
| <b>Henderson<br/>2008</b> <sup>62</sup>        | 50 mothers<br>of infants<br>born <34<br>weeks' PMA;<br>Australia              | Observational.<br>Adjusted for day<br>of expression and<br>GA                                                                                                          | From day one<br>to ten               | Milk yield<br>from day<br>one to ten                      | Significant association (no<br>effect estimate; p<0.001)                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                |                                                                               |                                                                                                                                                                        | ≥6/day from<br>day one to ten        |                                                           | Significant association (no<br>effect estimate)                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Hoban<br/>2018</b> <sup>274</sup>           | 16 mothers<br>of infants<br>born <33<br>weeks PMA;<br>USA                     | Observational.<br>No adjustment                                                                                                                                        | Day 14                               | Milk yield<br>≥500ml/d<br>by day 14<br>versus<br><500ml/d | Median 6.5 times/day (IQR<br>4-9) in high yield group<br>versus 4 times/day (IQR 3-<br>5) in low yield group.<br>p<0.001                                                                                                                                                                                                                                                                                                                          |
|                                                |                                                                               |                                                                                                                                                                        | ≥5/day at day<br>14 versus<br><5/day |                                                           | OR 21 (95% CI 1.5 – 293.2)<br>Calculated from data<br>reported                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Hill<br/>1999</b> <sup>39,43</sup>          | 39 mothers<br>of VPT &<br>VLBW<br>infants; USA                                | Observational.<br>Adjusted analysis<br>included<br>frequency of SSC,<br>income and<br>previous bf<br>experience                                                        | Week two to<br>five                  | Milk yield<br>≥500g/ day<br>in weeks<br>two to five       | Adjusted OR 1.15<br>(95% CI 1.01 – 1.30)                                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                |                                                                               |                                                                                                                                                                        | >6.1/day,<br>versus<br><6.1/day      | Milk yield<br>in weeks<br>two to five                     | High frequency group: 632<br>± 324g (n=19). Low<br>frequency group: 319 ±<br>292g (n=20)<br>MD = +313 mL (95% CI<br>119.1 - 506.9)                                                                                                                                                                                                                                                                                                                |



| Study                             | Context                                                                                         | Study method<br>Confounders                                                     | Expressing<br>frequency                                   | Outcome                            | Effect estimate                                                                                                                                                                                                                                                                                                                                                                                   |
|-----------------------------------|-------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|-----------------------------------------------------------|------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Lai 2020</b> <sup>40</sup>     | 25 mothers of infants born <34 weeks' PMA, still expressing by day eight after birth; Australia | Repeated measures observational Study. Adjusted for participant (random effect) | Day 10-20                                                 | Milk yield at day 10–20            | Linear mixed effects model coefficients (extracted from figure)<br>Expressing 4/day: 566g ± 131<br>Expressing 6/day: 784g ± 124<br>Expressing 8/day: 805g ± 135<br><br>Significant differences:<br>3 & 6/day: +228g (95% CI 43 - 414), p=0.006<br>4 & 5/day: +170g (95% CI 42 - 298), p=0.002<br>4 & 6/day: +223g (95% CI 77 - 370), p<0.001<br>No significant difference between 5 & up to 9/day |
| <b>Lussier 2015</b> <sup>41</sup> | 26 mothers of VPT & VLBW infants; USA                                                           | Observational. Adjusted for expressing method and day after birth               | First 28 days after birth                                 | Milk yield first 28 days           | MD 25 mL (95% CI 14 – 36)                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Morton 2009</b> <sup>273</sup> | 67 mothers of infants born <31 weeks' PMA and VLBW; USA                                         | Observational. No adjustment                                                    | Hand expressing >5/day on day one to three, versus <2/day | Milk yield during week two         | High frequency group: 710 ± 402 mL (n=16). Low frequency group: 392 ± 196 mL (n=15). MD = +318 mL (95% CI 97.5 – 538.5)                                                                                                                                                                                                                                                                           |
|                                   |                                                                                                 |                                                                                 | During week two                                           |                                    | Significant association (no effect estimate; p=0.008)                                                                                                                                                                                                                                                                                                                                             |
|                                   |                                                                                                 |                                                                                 | ≥7/day during week two versus <7/day                      |                                    | High frequency group: 622ml ± 424 (n=27). Low frequency group: 402 ± 238ml (n=35) p=0.03. MD = +220 mL (95% CI 41.7 – 398.3)                                                                                                                                                                                                                                                                      |
| <b>Murase 2014</b> <sup>277</sup> | 85 mothers of singleton VPT infants; Japan                                                      | Observational. Adjusted for maternal age and birth mode                         | Day one, three and four                                   | Milk yield on day four <153 mL/day | No significant association                                                                                                                                                                                                                                                                                                                                                                        |
|                                   |                                                                                                 |                                                                                 | Day two ≥4/day, versus <4/day                             | Milk yield on day 4                | OR 3.0 (95% CI 1.2 - 7.6) Adjusted OR 2.0 (95% CI 0.7 to 5.8)                                                                                                                                                                                                                                                                                                                                     |

| Study                                                              | Context                                                             | Study method<br>Confounders                                                                                   | Expressing<br>frequency                                                                                                                           | Outcome                                                       | Effect estimate                                                                                                                                                              |
|--------------------------------------------------------------------|---------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Ru 2020 <sup>36</sup>                                              | 30 mothers of VPT or VLBW infants; China                            | Observational. No adjustments                                                                                 | ≥6/day from birth to day 42, versus <6/day<br><br>[Including expressing frequency after the point of yield measurement is a concern for validity] | Milk yield at 14 days                                         | No significant association<br><br>768.5 ± 348.2 mL in high frequency group (n=26) versus 447.5 ± 361.3 mL in low frequency group (n=4). MD = +321 mL (95% CI -57.5 to 699.5) |
|                                                                    |                                                                     |                                                                                                               |                                                                                                                                                   | Milk yield at seven days                                      | No significant association<br><br>490.1 ± 231 mL in high frequency group (n=26) versus 299.5 ± 213.1 mL in low frequency group (n=4). MD = +267 mL (95% CI -102.2 to 636.2)  |
| Yu 2019 <sup>278</sup>                                             | 100 mothers of preterm infants admitted to NICU for ≥14 days; China | Observational. No adjustment                                                                                  | First 14 days after birth                                                                                                                         | Delayed secretory activation (>72 hours)                      | No significant association                                                                                                                                                   |
| <b>Outcome: Expressed milk yield more than 28 days after birth</b> |                                                                     |                                                                                                               |                                                                                                                                                   |                                                               |                                                                                                                                                                              |
| Fewtrell 2016 <sup>38</sup>                                        | 47 mothers of infants born <34 weeks' PMA; UK                       | Observational. Adjusted for GA, BW, milk yield at day 10, direct bf, double pump, pump comfort, hospital site | First ten days after birth                                                                                                                        | Milk yield whole admission                                    | No significant association                                                                                                                                                   |
| Hill 1999 <sup>39,43</sup>                                         | 39 mothers of VPT & VLBW infants; USA                               | Observational. Adjusted analysis included frequency of SSC, income, prior bf                                  | >6.4/day versus <6.4/day in weeks two to five                                                                                                     | Milk yield ≥500g/ day in weeks four to five versus <500g/ day | OR 8.1 (95% CI 1.8 - 37.1)<br><br>This was calculated from data reported                                                                                                     |
| Lau 2007 <sup>76</sup>                                             | 124 mothers of 'healthy' infants born 26 to 29 weeks' PMA; USA      | Observational. Adjusted for maternal depression, dose of SSC                                                  | Two, four, six, eight and ten weeks                                                                                                               | Milk yield on at two, four, six, eight and ten weeks          | Significant association (no effect estimate; p=0.001)                                                                                                                        |

| Study                                           | Context                                                                                                        | Study method<br>Confounders      | Expressing<br>frequency                             | Outcome                                     | Effect estimate                                                                                                                                                            |
|-------------------------------------------------|----------------------------------------------------------------------------------------------------------------|----------------------------------|-----------------------------------------------------|---------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Morton<br/>2009</b> <sup>273</sup>           | 67 mothers<br>of infants<br>born <31<br>weeks' PMA<br>& VLBW;<br>USA                                           | Observational.<br>No adjustment  | Week eight                                          | Milk yield<br>during<br>week eight          | Significant association (no<br>effect estimate; p=0.002)                                                                                                                   |
|                                                 |                                                                                                                |                                  | ≥7/day during<br>week eight<br>versus <7/day        |                                             | No significant association<br><br>High frequency group:<br>1019ml ± 686 (n=15). Low<br>frequency group: 752 ±<br>385ml (n=36). MD = +267<br>mL (95% CI -102.2 to<br>636.2) |
| <b>Ru 2020</b> <sup>36</sup>                    | 30 mothers<br>of VPT or<br>VLBW<br>infants;<br>China                                                           | Observational.<br>No adjustments | ≥6/day from<br>birth to day<br>42, versus<br><6/day | Milk yield<br>at 42 days                    | 1178.7 ± 419.4 mL in high<br>frequency group (n=26)<br>versus 557.5 ± 308.2 mL in<br>low frequency group<br>(n=4). MD = +621.2 mL<br>(95% CI 278.8 - 963.6)                |
| <b>Effect Estimate: Correlation coefficient</b> |                                                                                                                |                                  |                                                     |                                             |                                                                                                                                                                            |
| <b>Bishara<br/>2009</b> <sup>42</sup>           | 24 mothers<br>of EPT<br>infants<br>producing<br>≥120% of<br>infant intake<br>& expressing<br>≥4/day;<br>Canada | NA                               | Around week<br>three                                | Milk yield<br>around<br>week<br>three       | No significant correlation                                                                                                                                                 |
| <b>Fewtrell<br/>2016</b> <sup>38</sup>          | 62 mothers<br>of infants<br>born <34<br>weeks' PMA;<br>UK                                                      | NA                               | First ten days<br>after birth                       | Milk yield<br>first ten<br>days             | Pearson correlation<br>coefficient = 0.43                                                                                                                                  |
|                                                 |                                                                                                                |                                  |                                                     | Milk yield<br>whole<br>admission            | Pearson correlation<br>coefficient = 0.36                                                                                                                                  |
| <b>Gomez-<br/>Juge<br/>2023</b> <sup>275</sup>  | 31 mothers<br>of VPT or<br>VLBW<br>infants in<br>NICU for ≥3<br>weeks; USA                                     | NA                               | First week                                          | Milk yield<br>on day 7,<br>14, 21 and<br>28 | Correlation coefficient =<br>0.51 for day 21 outcome<br><br>No significant correlation<br>for day 7, 14 or 28<br>outcomes                                                  |
| <b>Hill<br/>2005</b> <sup>91,134,271</sup>      | 95 mothers<br>of VPT &<br>VLBW<br>infants; USA                                                                 | NA                               | Days six and<br>seven                               | Milk yield<br>in week 6                     | No significant correlation                                                                                                                                                 |
|                                                 |                                                                                                                |                                  | Day four                                            | Milk yield<br>≥500ml<br>/day week<br>6      | Spearman correlation<br>coefficient = 0.32                                                                                                                                 |
| <b>Hill<br/>1999</b> <sup>39,43</sup>           | 39 mothers<br>of VPT &<br>VLBW<br>infants; USA                                                                 | NA                               | Weeks two to<br>five                                | Milk yield<br>weeks two<br>to five          | Spearman correlation<br>coefficient = 0.33, 0.33,<br>0.32 and 0.35 respectively                                                                                            |

| Study                                   | Context                                                                       | Study method<br>Confounders | Expressing<br>frequency                   | Outcome                                                       | Effect estimate                                                              |
|-----------------------------------------|-------------------------------------------------------------------------------|-----------------------------|-------------------------------------------|---------------------------------------------------------------|------------------------------------------------------------------------------|
| <b>Hopkinson<br/>1988</b> <sup>44</sup> | 32 mothers<br>of infants<br>born 28 to<br>30 weeks'<br>PMA; USA               | NA                          | Week two and<br>week four                 | Milk yield<br>week two<br>and four                            | No significant correlation                                                   |
|                                         |                                                                               |                             | Week four                                 | Change in<br>milk yield<br>weeks two<br>to four               | Pearson correlation<br>coefficient = 0.49                                    |
| <b>Lau 2007</b> <sup>76</sup>           | 124 mothers<br>of 'healthy'<br>infants born<br>26 to 29<br>weeks' PMA;<br>USA | NA                          | Two, four, six,<br>eight and ten<br>weeks | Milk yield<br>at two,<br>four, six,<br>eight and<br>ten weeks | Significant correlation. No<br>coefficient reported                          |
| <b>Parker<br/>2020</b> <sup>253</sup>   | 69 mothers<br>of VPT &<br>VLBW<br>infants; USA                                | NA                          | Days two to<br>five                       | time to<br>secretory<br>activation                            | Spearman correlation<br>coefficient = between -0.3<br>and -0.42 for each day |

MOM = mother's own milk. VLBW = very low birth weight. LBW = low birth weight. VPT = very preterm. EPT = extremely preterm. PMA = post-menstrual age. CA = corrected age. OR = odds ratio. CI = confidence interval. IQR = interquartile range. GA = gestational age. BW = birthweight. SSC = skin to skin care (also called kangaroo care in some studies). Bf = breastfeeding.

## **Appendix I: Exploratory statistical analysis plan (related to Chapter 7)**

*Some of the following analyses are presented in the thesis. Others will be published separately as papers due to space constraints within the thesis.*

### **Objectives**

- Describe the expressing behaviours and human milk feeding outcomes of the trial population in detail
- Identify key variables associated with human milk feeding outcome, including identifying potential thresholds of expressed milk yield that could be used to identify mothers at high risk of poor outcome
- Identify key variables associated with expressed milk yield, on an individual expressing session and 24 hour level
- Identify key variables associated with other features of the trial dataset, such as adherence to expressing recommendations and mental health scores

### **Descriptive analysis**

The nature of the expressing and breastfeeding experience will be described using summary statistics and graphical representations. Identification of delayed lactogenesis II will use an established volume based definition (on day 4, absence of two consecutive expressing sessions with >20g milk at each session<sup>1</sup>; or 24 hour yield <40g if only one session logged)

### **Predicting breastfeeding outcomes**

The outcome of this analysis is breastfeeding behaviour (binary outcome). The outcome of any versus no breastfeeding will be assessed at 4 months corrected age. The outcome of exclusive breastfeeding versus any formula feeding will be assessed at 36 weeks PMA and 2 months corrected age.

Univariate and then multivariable analysis will take place with the explanatory variable of 24-hour yield. Associations with a p value >0.15 will be considered for inclusion in multivariable analysis, along with a

priori identified important potential confounders and independent variables identified through literature review and DAG construction. Given the limited power of the sample, a guideline of no more than one variable per 5 outcome events (e.g. non-exclusive HMF) will be used – this may require separate regression models for groups of variables. Related variables with high association may also be tested in clusters to identify the most important to include in modelling, such as socioeconomic variables; expressing dynamic variables and so on. Multivariable analysis will use logistic regression. Repeated measures techniques will be used, such as clustering by individual.

ROC analysis will take place to explore potentially clinically meaningful yield thresholds at different timepoints, both unadjusted and adjusted. A priori threshold for a useful clinical threshold are sensitivity of 80% and specificity of 70%. Thresholds identified in previous literature will also be assessed.

Latent class analysis will be used to identify latent classes of yield trajectory. Such classes may then contribute to multivariable analysis.

### **Dynamics of expressing yield**

The outcome of this analysis is 24-hour yield (continuous outcome). Univariate and then multivariable analysis will take place with the explanatory variable of expressing frequency. Choice of variable strategy will be as above. Multivariable analysis will use linear regression. Repeated measures techniques will be used, such as clustering by individual. An interaction term of expressing frequency and time to first ever expression will be tested. Time to first ever expression will be modelled as a continuous variable and a categorical variable using clinically meaningful thresholds. Interaction terms of time with other variables such as yield may be required. Factors associated with any latent classes of yield trajectory may also be explored, using logistic regression.

The yield and rate of milk synthesis (milk yield divided by the time since last expression) will be modelled using linear regression at an individual expressing session level, using key variables of duration of expression session, expressing frequency, expressing mode and time since last expression – clustered

on individual. Quadratic modelling will be compared to linear modelling for best fit, due to previous literature suggesting a quadratic relationship.

### **Other questions**

Linear and logistic regression will be used to analyse the relationship of baseline variables with intervention compliance; the relationship of baseline variables with expressing recommendations (timing of first expression after birth; expressing frequency); the relationship of yield with maternal perception of yield; and the predictive factors for maternal anxiety and post-traumatic stress reactions (assessed using STAI and PCL-5 psychometric scores).

### **Notes for model building and choice of thresholds for categorisation**

Previous literature suggests the following variables are important for milk yield:

- Mothers age
- Mothers education level
- Mothers income
- Mothers previous breastfeeding experience
- Mothers breastfeeding intention
- Gestation (variable direction of effect)
- Time to first expression (studies have used 1, 2, 3, 6, 8, 24, 48 hour cut offs. Current clinical recommendations generally use 2 and 6 hour cut offs)
- Expressing frequency (depending on timepoint – no relationship seen at later timepoints)
- Skin to skin dose
- Number of direct breastfeeds/time at breast
- Double pumping
- Yield at earlier timepoint

In addition, previous literature suggests the following are important for breastfeeding outcome:

- Multiple birth
- Infant gender
- Length of stay
- Birthweight
- Infant complications
- Maternal ethnicity
- Caesarean birth
- Expressing yield
  - Several studies reported outcomes by binary yield cut offs without clear explanation of why those cut offs were chosen or because of expert opinion, these are:
    - 500ml at day 10/14; 750ml at day 30; 153ml on day 4
  - Several studies defined specific thresholds using ROC, these are:
    - 407g on day 7 (to predict later yield of >750g)
    - 518g on day 14 (to predict later yield of >750g)
    - 190ml on day 7 (to predict discharge yield >300ml)



## Appendix J: Tables supporting the exploratory trial results (Chapter 7)

**Table J.13: Breastmilk feeding outcomes over time**

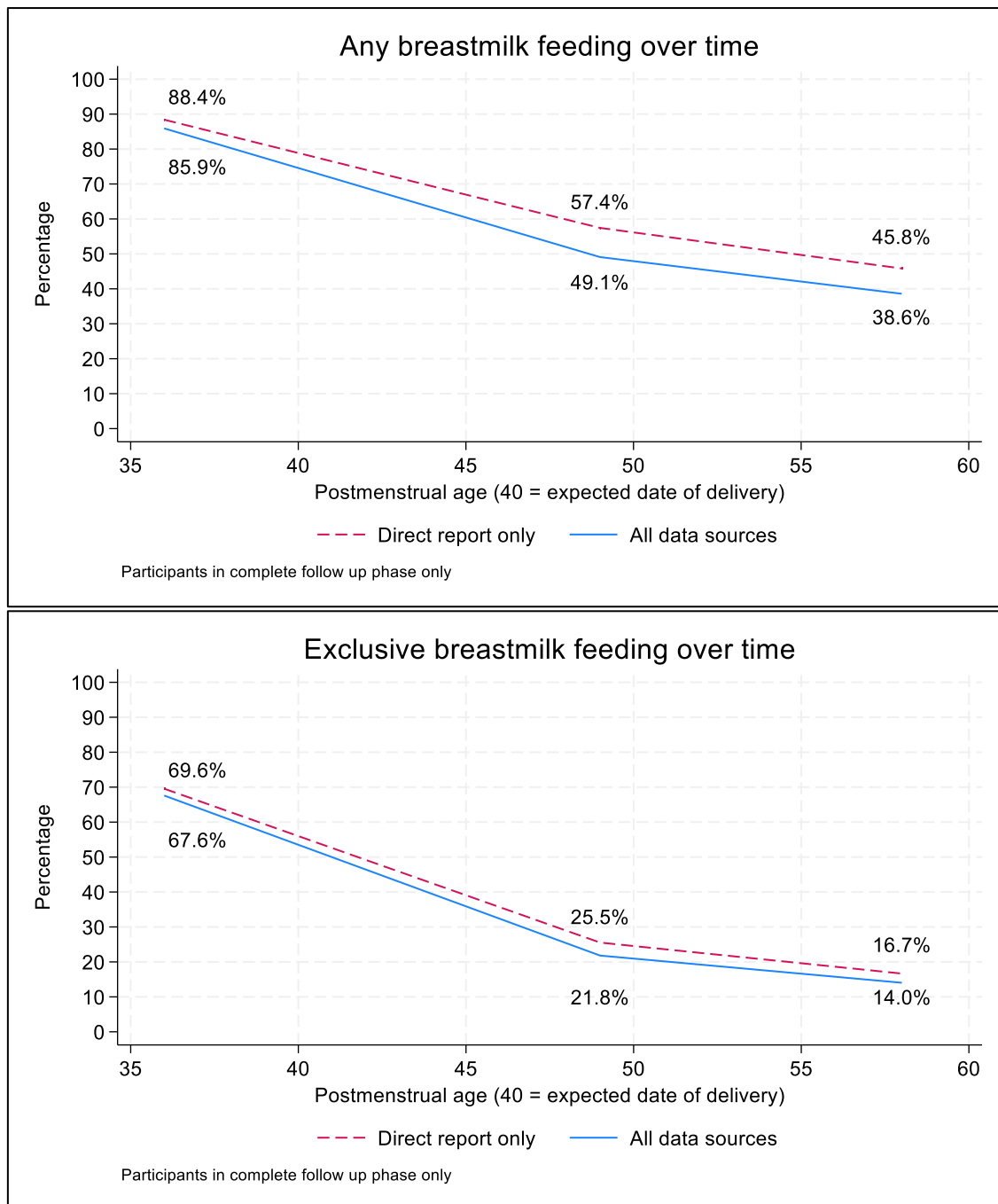
|                                                               | Exclusive breastmilk |                    | Any breastmilk    |                    | Infant death <sup>†</sup> | Missing <sup>†</sup> |
|---------------------------------------------------------------|----------------------|--------------------|-------------------|--------------------|---------------------------|----------------------|
|                                                               | All data sources     | Direct report only | All data sources  | Direct report only |                           |                      |
| <b>All participants<sup>64</sup> n/N (%)</b>                  |                      |                    |                   |                    |                           |                      |
| <b>36 weeks' PMA</b>                                          | 74/114<br>(64.9%)    | 74/110<br>(67.3%)  | 98/114<br>(86.0%) | 98/110<br>(89.1%)  | 6                         | 12                   |
| <b>Long term follow up phase<sup>65</sup>; n/N (%)</b>        |                      |                    |                   |                    |                           |                      |
| <b>36 weeks' PMA</b>                                          | 48/71<br>(67.6%)     | 48/69<br>(69.6%)   | 61/71<br>(85.9%)  | 61/69<br>(88.4%)   | 4                         | 10                   |
| <b>Two months' CA</b><br>(49 weeks' PMA)                      | 12/55<br>(21.8%)     | 12/47<br>(25.5%)   | 27/55<br>(49.1%)  | 27/47<br>(57.4%)   | 4                         | 26                   |
| <b>Four months' CA</b><br>(58 weeks' PMA)                     | 8/57<br>(14.0%)      | 8/48<br>(16.7%)    | 22/57<br>(38.6%)  | 22/48<br>(45.8%)   | 5                         | 23                   |
| <b>Long term follow up phase &amp; complete data; n/N (%)</b> |                      |                    |                   |                    |                           |                      |
| <b>36 weeks' PMA</b>                                          | 34/53<br>(64.2%)     | 34/43<br>(79.1%)   | 43/53<br>(81.1%)  | 42/43<br>(97.7%)   |                           |                      |
| <b>Two months' CA</b><br>(49 weeks' PMA)                      | 12/53<br>(22.6%)     | 12/43<br>(27.9%)   | 26/53<br>(49.1%)  | 26/43<br>(60.5%)   |                           |                      |
| <b>Four months' CA</b><br>(58 weeks' PMA)                     | 8/53<br>(15.1%)      | 8/43<br>(18.6%)    | 20/53<br>(37.7%)  | 20/43<br>(46.5%)   |                           |                      |

*\*Includes no enteral feeding. Included in denominators. <sup>†</sup>Not included in denominators. PMA = post-menstrual age. CA = corrected age. Direct report only refers to participant response to SMS or extraction from infant medical notes. All data sources also includes report of breastfeeding cessation at previous timepoint*

<sup>64</sup>132 eligible

<sup>65</sup>85 eligible

**Figure J.3: Comparing breastmilk prevalence with different sources of data**



*Direct report only refers to participant response to SMS or extraction from infant medical notes. All data sources also includes report of breastfeeding cessation at previous timepoint*

**Table J.14: Baseline characteristics and expressed milk yield (day 4 and day 21)**

|                                                     | 24-hour milk yield day 4 (g) |                 |          | 24-hour milk yield day 21 (g) |                  |          |
|-----------------------------------------------------|------------------------------|-----------------|----------|-------------------------------|------------------|----------|
|                                                     | Mean (SD)                    | Median [IQR]    | <i>n</i> | Mean (SD)                     | Median [IQR]     | <i>n</i> |
| <b>Ethnic Background</b>                            |                              |                 |          |                               |                  |          |
| Asian                                               | 173.7 (165.6)                | 118 [60 – 288]  | 18       | 547.6 (367.2)                 | 469 [302 – 796]  | 14       |
| Black                                               | 258.5 (253.0)                | 127 [66 – 500]  | 19       | 652.5 (498.9)                 | 684 [170 – 946]  | 17       |
| White                                               | 229.7 (213.7)                | 156 [66 – 357]  | 58       | 542.1 (366.8)                 | 485 [243 – 778]  | 55       |
| Other                                               | 183.8 (42.2)                 | 187 [157 – 211] | 4        | 496.4 (189.5)                 | 499 [305 – 684]  | 3        |
| <b>Index of multiple deprivation (IMD) quintile</b> |                              |                 |          |                               |                  |          |
| 1 (most deprived)                                   | 248.1 (229.7)                | 156 [118 – 296] | 18       | 516.3 (467.9)                 | 391 [305 – 567]  | 13       |
| 2                                                   | 205.3 (244.9)                | 85 [19 – 304]   | 23       | 460.1 (414.5)                 | 388 [107 – 796]  | 17       |
| 3                                                   | 176.8 (104.2)                | 180 [105 – 246] | 16       | 561.8 (269.8)                 | 554 [290 – 733]  | 15       |
| 4                                                   | 257.7 (229.1)                | 168 [87 – 392]  | 20       | 644.9 (387.6)                 | 623 [314 – 968]  | 20       |
| 5 (least deprived)                                  | 214.6 (196.2)                | 128 [51 – 359]  | 24       | 583.3 (391.8)                 | 575 [239 – 938]  | 25       |
| <b>Maternal age</b>                                 |                              |                 |          |                               |                  |          |
| <25 years                                           | 212.8 (221.7)                | 136 [85 – 190]  | 10       | 435.2 (395.4)                 | 363 [295 – 405]  | 6        |
| 25 to <30 years                                     | 208.4 (168.0)                | 208 [71 – 346]  | 17       | 536.9 (368.8)                 | 477 [272 – 821]  | 16       |
| 30 to <35 years                                     | 228.9 (215.7)                | 186 [71 – 288]  | 45       | 573.5 (362.5)                 | 529 [252 – 848]  | 42       |
| ≥35 years                                           | 218.9 (223.0)                | 127 [51 – 276]  | 29       | 582.7 (445.1)                 | 490 [232 – 778]  | 26       |
| <b>Full time education ceased</b>                   |                              |                 |          |                               |                  |          |
| 16 years                                            | 294.3 (275.0) <sup>†</sup>   | 147 [85 – 548]  | 10       | 381.8 (321.7)*                | 291 [211 – 405]  | 10       |
| 17-18 years                                         | 155.0 (105.2)                | 157 [59 – 231]  | 19       | 328.7 (250.5)                 | 269 [164 – 495]  | 16       |
| ≥19 years                                           | 231.5 (218.0)                | 153 [66 – 357]  | 70       | 650.6 (395.3)                 | 655 [318 – 946]  | 63       |
| <b>Presence of partner</b>                          |                              |                 |          |                               |                  |          |
| No partner                                          | 155.5 (198.9)                | 85 [10 – 192]   | 10       | 466.5 (362.3)                 | 376 [206 – 846]  | 7        |
| <b>Caesarean birth</b>                              |                              |                 |          |                               |                  |          |
| Vaginal birth                                       | 246.2 (221.6)                | 208 [66 – 364]  | 43       | 541.0 (326.3)                 | 488 [291 – 768]  | 36       |
| <b>Multiple birth</b>                               |                              |                 |          |                               |                  |          |
| Singleton birth                                     | 281.4 (260.0)                | 231 [54 – 546]  | 16       | 723.0 (502.7) <sup>†</sup>    | 618 [318 – 1121] | 14       |
| Singleton birth                                     | 209.6 (196.9)                | 151 [70 – 288]  | 85       | 530.5 (357.1)                 | 487 [245 – 810]  | 76       |
| <b>Primiparous</b>                                  |                              |                 |          |                               |                  |          |
| Multiparous                                         | 183.4 (159.8) <sup>†</sup>   | 138 [68 – 251]  | 60       | 509.7 (327.1) <sup>†</sup>    | 485 [290 – 733]  | 53       |
| Multiparous                                         | 282.2 (256.3)                | 185 [77 – 447]  | 40       | 633.1 (453.1)                 | 597 [243 – 938]  | 37       |
| <b>Gestation at birth</b>                           |                              |                 |          |                               |                  |          |
| 23 to <26wk                                         | 216.7 (245.3)                | 122 [23 – 276]  | 27       | 494.6 (361.9)                 | 374 [211 – 758]  | 22       |
| 26 to <28wk                                         | 243.4 (218.9)                | 186 [71 – 364]  | 29       | 616.5 (467.7)                 | 521 [205 – 978]  | 24       |
| 28 to <30wk                                         | 203.8 (211.4)                | 137 [86 – 226]  | 22       | 638.7 (367.4)                 | 567 [376 – 860]  | 23       |
| 30 to <32wk                                         | 214.3 (146.7)                | 190 [113 – 304] | 23       | 479.7 (322.9)                 | 391 [243 – 702]  | 21       |
| <b>Ventilated at baseline</b>                       |                              |                 |          |                               |                  |          |
| Not ventilated at baseline                          | 182.3 (207.3) <sup>†</sup>   | 108 [43 – 259]  | 36       | 484.5 (390.0) <sup>†</sup>    | 326 [206 – 796]  | 31       |
| Not ventilated at baseline                          | 242.4 (207.4)                | 192 [86 – 304]  | 65       | 600.4 (381.9)                 | 554 [295 – 848]  | 59       |
| <b>Exclusive breastmilk intention</b>               |                              |                 |          |                               |                  |          |
| No exclusive intention                              | 232.8 (215.1)                | 181 [70 – 342]  | 70       | 600.4 (418.4) <sup>†</sup>    | 506 [248 – 882]  | 65       |
| No exclusive intention                              | 199.8 (193.7)                | 152 [66 – 242]  | 30       | 456.5 (266.6)                 | 408 [287 – 684]  | 25       |
| <b>Longest previous breastmilk feeding</b>          |                              |                 |          |                               |                  |          |
| <26 weeks (6 months)                                | 170.9 (200.2)*               | 123 [23 – 209]  | 19       | 431.0 (326.4)*                | 302 [209 – 758]  | 15       |
| ≥26 weeks (6 months)                                | 382.9 (263.9)                | 367 [151 – 609] | 21       | 770.9 (481.5)                 | 719 [413 – 1126] | 22       |

|                                     | 24-hour milk yield day 4 (g) |                |          | 24-hour milk yield day 21 (g) |                  |          |
|-------------------------------------|------------------------------|----------------|----------|-------------------------------|------------------|----------|
|                                     | Mean (SD)                    | Median [IQR]   | <i>n</i> | Mean (SD)                     | Median [IQR]     | <i>n</i> |
| <b>Baseline clinical anxiety</b>    |                              |                |          |                               |                  |          |
| STAI score >40                      | 203.9 (192.6)                | 148 [59 – 256] | 69       | 520.0 (355.5) <sup>†</sup>    | 474 [248 – 778]  | 61       |
| STAI score ≤40                      | 251.7 (231.2)                | 197 [78 – 335] | 24       | 647.9 (454.1)                 | 575 [243 – 882]  | 23       |
| <b>Recruitment Trust</b>            |                              |                |          |                               |                  |          |
| Oxford                              | 226.8 (210.9)                | 139 [66 – 361] | 59       | 551.1 (398.0)                 | 499 [243 – 830]  | 61       |
| Imperial                            | 205.6 (191.6)                | 185 [51 – 266] | 32       | 636.4 (339.3)                 | 491 [376 – 882]  | 21       |
| Manchester                          | 235.9 (260.6)                | 152 [71 – 242] | 10       | 432.2 (417.9)                 | 307 [145 – 640]  | 8        |
| <b>First expression after birth</b> |                              |                |          |                               |                  |          |
| ≤2 hours                            | 283.9 (273.4) <sup>†</sup>   | 249 [23 – 477] | 19       | 663.9 (407.1)                 | 628 [248 – 1126] | 18       |
| >2 to 6 hours                       | 248.6 (214.4)                | 209 [71 – 357] | 33       | 555.39 (416.1)                | 467 [291 – 827]  | 32       |
| >6 hours                            | 173.2 (160.0)                | 129 [66 – 239] | 47       | 520.8 (356.3)                 | 474 [232 – 778]  | 39       |

\* $p < 0.05$  <sup>†</sup> $p < 0.2$  (Rank sum or *t* test)

**Table J.15: Adjusted association of expressing session variables with hourly milk yield at day 4, 14 and 21**

|                                                               | Day 4                              |         | Day 14                             |         | Day 21                             |         |
|---------------------------------------------------------------|------------------------------------|---------|------------------------------------|---------|------------------------------------|---------|
|                                                               | Hourly milk yield in g/hr (95% CI) | p value | Hourly milk yield in g/hr (95% CI) | p value | Hourly milk yield in g/hr (95% CI) | p value |
| Gap to prior session (hours)                                  | -0.9 (-1.1 to -0.7)                | <0.001  | -2.1 (-2.5 to -1.7)                | <0.001  | -2.7 (-3.3 to -2.1)                | <0.001  |
| Session duration (per 10 mins)                                | 1.1 (0.2 to 1.9)                   | 0.01    | -                                  |         | -                                  |         |
| Electric pump only (compared to hand, manual or mixture)      | 7.1 (4.4 to 9.9)                   | <0.001  | -                                  |         | -                                  |         |
| Simultaneous expression (compared to single or sequential)    | 4.8 (0.7 to 8.8)                   | 0.02    | -                                  |         | -                                  |         |
| First expression ≤6 hours from birth                          | 4.7 (1.5 to 8.0)                   | 0.004   | -                                  |         | -                                  |         |
| Previous bf experience ≥6mth (compared to <6mth /primiparous) | 9.8 (5.8 to 13.9)                  | <0.001  | 20.2 (13.4 to 26.9)                | <0.001  | 16.6 (9.0 to 24.2)                 | <0.001  |
| Maternal age (per 10 years)                                   | -2.6 (-5.6 to 0.3)                 | 0.08    |                                    |         | -6.2 (-12.7 to 0.4)                | 0.07    |
| Multiple birth                                                | -                                  |         | 8.8 (1.4 to 16.2)                  | 0.02    | 6.8 (-1.6 to 15.2)                 | 0.11    |
| Left full time education ≥18 years                            | -                                  |         |                                    |         | 5.1 (-2.2 to 12.3)                 | 0.17    |
| Intention to exclusively breastmilk feed                      | -                                  |         |                                    |         | 6.1 (-0.8 to 13.0)                 | 0.09    |

**Table J.16: Sensitivity analysis: removal of participants with incomplete logs**

| Day 4                       |                                      |         |    |
|-----------------------------|--------------------------------------|---------|----|
|                             | 24-hour milk yield in grams (95% CI) | p value | n  |
| <b>Expressing frequency</b> |                                      |         |    |
| ≥8/day                      | [baseline]                           |         | 13 |
| <8/day                      | -131.8 (-235.8 to -27.8)             | 0.01    | 67 |
| <b>Expressing frequency</b> |                                      |         |    |
| ≥8/day                      | [baseline]                           |         | 13 |
| 6-7/day                     | -95.9 (-211.7 to 19.9)               | 0.10    | 26 |
| <6/day                      | -154.5 (-262.9 to -46.0)             | 0.006   | 41 |
| <b>Longest gap</b>          |                                      |         |    |
| <6 hours                    | [baseline]                           |         | 24 |
| ≥6/day                      | -90.0 (-175.5 to -4.8)               | 0.04    | 56 |
| <b>Longest gap</b>          |                                      |         |    |
| <6 hours                    | [baseline]                           |         | 24 |
| 6-7 hours                   | -55.9 (-158.5 to 46.7)               | 0.28    | 22 |
| ≥8 hours                    | -113.1 (-206.6 to -19.6)             | 0.02    | 34 |

**Table J.17: Sensitivity analysis: removal of one participant outlier for milk yield**

|                             | Day 4                                |         |    | Day 21                               |         |    |
|-----------------------------|--------------------------------------|---------|----|--------------------------------------|---------|----|
|                             | 24-hour milk yield<br>grams (95% CI) | p value | n  | 24-hour milk yield<br>grams (95% CI) | p value | n  |
| <b>Expressing frequency</b> |                                      |         |    |                                      |         |    |
| ≥8/day                      | [baseline]                           |         | 13 | [baseline]                           |         | 14 |
| <8/day                      | -103.0 (-201.8 to -4.1)              | 0.04    | 89 | -192.4 (-394.3 to 9.5)               | 0.06    | 76 |
| <b>Expressing frequency</b> |                                      |         |    |                                      |         |    |
| ≥8/day                      | [baseline]                           |         | 13 | [baseline]                           |         | 14 |
| 6-7/day                     | -62.2 (-171.5 to 47.0)               | 0.26    | 30 | -92.5 (-296.3 to 111.3)              | 0.37    | 41 |
| <6/day                      | -125.0 (-226.4 to -23.6)             | 0.02    | 59 | -325.2 (-537.0 to -113.5)            | 0.003   | 35 |
| <b>Longest gap</b>          |                                      |         |    |                                      |         |    |
| <6 hours                    | [baseline]                           |         | 26 | [baseline]                           |         | 35 |
| ≥6/day                      | -78.9 (-155.8 to -2.0)               | 0.04    | 75 | -218.2 (-357.4 to -78.9)             | 0.003   | 51 |
| <b>Longest gap</b>          |                                      |         |    |                                      |         |    |
| <6 hours                    | [baseline]                           |         | 26 | [baseline]                           |         | 35 |
| 6-7 hours                   | -28.2 (-123.9 to 67.5)               | 0.56    | 23 | -190.2 (-359.2 to -21.2)             | 0.03    | 23 |
| ≥8 hours                    | -101.8 (-182.2 to -21.3)             | 0.01    | 52 | -263.8 (-425.2 to -102.5)            | 0.002   | 28 |

**Table J.18: Sensitivity analysis: limiting assumptions of derivation of the gap prior to first logged sessions**

|                    | Day 4                                |         |    | Day 21                               |         |    |
|--------------------|--------------------------------------|---------|----|--------------------------------------|---------|----|
|                    | 24-hour milk yield<br>grams (95% CI) | p value | n  | 24-hour milk yield<br>grams (95% CI) | p value | n  |
| <b>Longest gap</b> |                                      |         |    |                                      |         |    |
| <6 hours           | [baseline]                           |         | 48 | [baseline]                           |         | 55 |
| ≥6 hours           | -7.1 (-78.8 to 64.6)                 | 0.85    | 44 | -129.0 (-294.1 to 36.1)              | 0.12    | 29 |
| <b>Longest gap</b> |                                      |         |    |                                      |         |    |
| <6 hours           | [baseline]                           |         | 48 | [baseline]                           |         | 55 |
| 6-7 hours          | 54.1 (-27.0 to 135.1)                | 0.19    | 25 | -179.8 (-374.2 to 14.7)              | 0.07    | 16 |
| ≥8 hours           | -93.3 (-183.7 to -2.9)               | 0.04    | 19 | -139.8 (-353.6 to 74.1)              | 0.20    | 13 |

## Appendix K: Dissemination materials related to the exploratory findings (Chapter 7):

