EXPRESS
EXpressing in PREmaturity - Simple interventionS

Study Title: Does a guided relaxation audio track increase yield of expressed milk in mothers of very preterm infants? A randomised controlled trial and nested exploratory work

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Conflicts of Interest:
Ilana Levene is a trustee of a community breastfeeding support charity in Oxfordshire, and Co-Chair of the Hospital Infant Feeding Network, promoting breastfeeding in hospital settings.

Maria Quigley has no conflicts of interest to declare.

Mary Fewtrell receives an unrestricted research donation from Philips for research in infant nutrition. She is Clinical Lead for Nutrition, Royal College of Paediatrics & Child Health, UK and a member of the infant nutrition working group, European Food Safety Authority (EFSA).

Frances O'Brien has no conflicts of interest to declare.

Confidentiality Statement
This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

ISRCTN Trial Registration Number: 16356650
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Protocol signature page
The undersigned has read and understood the trial protocol detailed below and agrees to conduct the trial in compliance with the protocol.

Principal Investigator	Signature	Site name or ID number	Date

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2. LAY SUMMARY

Babies who are born early (prematurely) are more likely to have serious health problems, which can affect them for the rest of their lives. Being born early is also the most common cause of death for babies in the UK. Parents are more likely to be anxious and depressed when their baby is born early. We know that giving breastmilk to premature babies is very important for their health, but this isn't always easy for mothers. Being anxious may make it more difficult to produce breastmilk.

This is a trial for women who gave birth very early – when they were less than 7 months pregnant. It aims to increase the amount of breastmilk mothers can produce, which in turn will improve their chances of breastfeeding their babies when they are well enough to go home.

Babies who are born this early can't breastfeed directly, instead they have milk put into their stomach through a tube. The ideal milk to give to premature babies is breastmilk. Breastmilk prevents serious gut illnesses, infections and eye problems, and builds babies' brains for their long-term development.

Mothers are advised to express milk from their breasts, by hand or using a pump, to give to their very premature babies. However, it can be hard for mothers who give birth this early to produce and express enough breastmilk for their baby. This may limit their long-term milk production capacity and therefore prevent them from exclusively breastfeeding when their baby is strong enough.

This trial will recruit a group of mothers and randomly allocate half to listen to a relaxation and visualisation soundtrack while expressing milk and half to normal care while expressing milk. The soundtrack will talk the mother through relaxing their muscles and also picturing their baby and imagining their milk flowing. We want to see if listening to the soundtrack increases the amount of milk mothers can express in the first three weeks after birth. We want to see if the soundtrack will help more mothers to exclusively breastfeed around the time their baby goes home. We will also look at whether listening to the soundtrack helps mothers feel less anxious or distressed.

3. SYNOPSIS

Study Title	Does a guided relaxation audio track increase yield of expressed milk in mothers of very preterm infants? A randomised controlled trial and nested exploratory work	
Internal ref. no. / short title	EXPRESS (EXpressing in PREmaturity – Simple interventionS)	
Study registration	ISRCTN 16356650 19/04/2021	
Sponsor	University of Oxford Research Governance, Ethics & Assurance, Research Services, Joint Research Office, Boundary Brook House, Churchill Drive, Oxford, OX3 7GB	
Funder	National Institute for Health Research (NIHR) NIHR Academy, 21 Queen Street, Leeds LS1 2TW elizabeth.taylor@nihr.ac.uk 01135328407	
Study Design	Parallel group randomised controlled trial	
Study Participants	Mothers of babies born at 23^{+0} to 31^{+6} weeks of pregnancy and intending to express milk for at least 14 days	
Planned Sample Size	132 mothers (1:1 allocation ratio)	
Planned Study Period	Total data collection period: 22 months Trial recruitment period: 19 months Length of individual participants' involvement: one to eight months (dependent on level of prematurity and whether enrolled in long term follow up)	
	Objectives	**Outcome Measures/Timepoint**
Primary	Increase expressed milk yield	24-hour weight of expressed milk (highest value recorded at any of the study timepoints up to and including day 21)
Secondary	Increase the achievement of Unicef Baby Friendly Initiative target expressed milk yield	Proportion expressing at least 750g during any of the 24-hour study timepoints up to and including day 21
	Increase expressing efficiency	Average weight of milk expressed per minute at day 21
	Reduce anxiety	Average anxiety score (STAI-6) on day 21

	Reduce distress	Average stress reaction score (PCL-5) on day 21
	Increase exclusive human milk feeding	Proportion exclusive human milk feeding at 36 weeks' post-menstrual age
		Proportion exclusive human milk feeding at 18 weeks' corrected age (18 weeks after the EDD)
	Increase any human milk feeding	Proportion with any human milk feeding at 36 weeks' post-menstrual age
Process Indicators	Assess the effect on time spent in skin to skin contact with infant	Average number of hours spent in skin to skin contact with infant/s at day 21
	Assess the effect on frequency of expressing	Average number of expressing episodes in 24 hours at day 21
	Assess the effect on time spent expressing	Average number of hours spent expressing in 24 hours at day 21
Intervention	Standard care plus guided visualisation and muscle relaxation audio track. Participants asked to listen to the track while expressing as often as possible for 21 days	
Comparator	Standard care	

4. ABBREVIATIONS

ANOVA	Analysis of Variance
BFI	Baby Friendly Initiative
BQ	Baseline Questionnaire
CI	Chief Investigator
CRF	Case Report Form
DSM-5	Diagnostic and Statistical Manual of Mental Disorders - Fifth Edition
EDD	Estimated Date of Delivery
GCP	Good Clinical Practice
HMF	Human milk feeding
HRA	Health Research Authority
MER	Milk Ejection Reflex
MOM	Mother's Own Milk

NEC	Necrotising Enterocolitis
NHS	National Health Service
NICU	Neonatal Intensive Care Unit
NNAP	National Neonatal Audit Programme
PCL-5	Post-traumatic stress Checklist for DSM-5
PIS	Participant Information Sheet
PI	Principal Investigator
PIL	Participant/Patient Information Leaflet
PMA	Post-menstrual age
PPI	Patient Public Involvement
PTSD	Post-traumatic Stress Disorder
R&D	NHS Trust R&D Department
RCT	Randomised controlled trial
REC	Research Ethics Committee
RES	Research Ethics Service
ROC	Receiver Operating Characteristics
ROP	Retinopathy of Prematurity
SOP	Standard Operating Procedure
STAI	State-Trait Anxiety Inventory
VLBW	Very Low Birth Weight

4.1. Specialist terminology and knowledge

The prematurity of an infant is expressed by the number of complete weeks + number of complete days that have passed since the first day of the mother's last menstrual period – this is their post-menstrual age (PMA). For example, an infant might be born at 25+2 PMA (25 weeks and 2 days since the first day of the mother's last menstrual period). At 2 days old this infant would be 25+4 PMA. 40 weeks' PMA is the infant's original due date or estimated date of delivery (EDD). After 40 weeks' PMA the infant may be referred to by their corrected age – for example at one month after the EDD the infant is one month corrected age. This is differentiated from the infant's actual age – the time that has elapsed since their birth.

The day that a baby is born is termed 'Day 0'. 'Day 1' of life starts at 00:01 on the calendar date following the day of birth. For example, if the mother gives birth on 24th March, day 1 starts at 00:01 on 25th March, regardless of the time of the baby's birth.

The infants of all potential participants in this study would be admitted to a neonatal unit at birth. Expected care would include an incubator, significant monitoring and a gradual introduction of milk feeds through a tube into the stomach. Each infant has a unique pathway of being ready for

milk feeds by mouth, but this generally starts around 34-36 weeks' PMA. Expected discharge date is also dependent on each infant's unique pathway and gestational age. As an overall average, infants are discharged around their EDD but there is a large variation.

5. BACKGROUND AND RATIONALE

5.1. Background

There are approximately 11,500 extremely preterm (born at <28 weeks' PMA) and very preterm births (born at 28–31 weeks' PMA) each year in England and Wales[1]. Complications arising from premature birth are the leading cause of neonatal death in the UK[2], and the cost of preterm birth to the public sector is over £3 billion considering childhood alone[3]. Babies born preterm have high rates of early, late and post neonatal mortality, and the risk of mortality increases as gestational age at birth decreases. Babies who survive have increased rates of disability[2].

In addition, premature birth has a significant effect on parental mental health – 80% of parents report a deterioration in their mental health after their baby's neonatal unit admission[4]. In mothers of very preterm babies two weeks after delivery, 17% could be diagnosed with clinical anxiety, 28% with depression and 52% with post-traumatic stress reactions – notably mothers of babies who were particularly unwell were excluded from this study, underestimating the true incidence[5]. A systematic review reported rates of depression between 29% and 40% in mothers of very preterm or very low birth weight babies in the first month of life, which is a significantly higher incidence than for mothers of term babies[6].

5.2. Mother's own milk

Infants born very premature do not have the oral skills to directly breastfeed but are given milk directly into the stomach. Receiving the maximum amount of mother's own milk (MOM) in the first few weeks after birth is a vital intervention to lessen morbidity and mortality in preterm babies. For example, very low birth weight (VLBW) babies who receive less than half their nutrition as MOM in the first 10 days of life are 60% more likely to die, have NEC (a potentially lethal gut infection) or other serious infection[7]. Providing exclusive MOM to only 21 babies, in comparison to any formula, would prevent one case of severe retinopathy of prematurity (ROP, one of the commonest causes of childhood blindness[8]). Increasing human milk also incrementally improves brain growth[9] and long-term neurodevelopmental outcomes[9,10]. It is therefore clear that maximising early and continued MOM for each preterm baby has a powerful impact on their chances of survival and their long-term health. The importance of early MOM in prematurity is shown by its inclusion (mothers' milk on day 14 after birth) as a new outcome in 2020 in the National Neonatal Audit Programme (NNAP), a national benchmarking process to evaluate the quality of neonatal care[11].

Maximising early MOM yield also leads to better long-term human milk feeding (HMF) outcomes[12,13], with all the consequent benefits in terms of reduction in sudden infant death syndrome, childhood infections, childhood obesity, maternal cancer and many more[14]. In very preterm babies each 10ml/kg/day of MOM that the baby receives at day 7 gives a 20% higher chance of exclusive HMF at 36 weeks' PMA[15], and for every 10% increase in MOM provided during the neonatal stay, there is a 93% increase in likelihood of HMF at discharge[16]. The

importance of discharge HMF in prematurity is shown by its inclusion as one of only 19 core outcomes measured by the NNAP to evaluate the quality of all neonatal care[11].

Lack of MOM also impacts on NHS costs – VLBW infants with the lowest tertile of MOM intake have 22% higher overall NICU costs (corrected for other factors) than those in the highest tertile[17]. Increasing any HMF in babies at NICU discharge 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[18].

5.3. Poor milk supply

Despite these compelling reasons to provide maximal MOM to preterm babies at the earliest possible time, mothers of preterm babies have a high risk of poor milk supply, leading to non-exclusive human milk feeding[19–23]. 40% of babies born in the UK at <33 weeks' PMA were exclusively formula fed at discharge home in 2018, and this figure has not improved in the last five years[11]. The challenges are multiple – very preterm babies cannot directly breastfeed due to their immaturity, so mothers must establish and then maintain their milk supply by mechanical expression, which they find challenging[24,25]. Breast tissue is relatively underdeveloped at delivery and lactational hormonal cycles not fully established when babies are born prematurely. Antenatal steroids, vital for preterm babies' lung development, delay milk 'coming in'[26] (lactogenesis II). Importantly, emotional distress and anxiety inhibit the milk ejection reflex (MER) or 'let-down', which is necessary for significant milk flow and establishment of milk supply[27]. These factors combine to contribute to high levels of insufficient milk supply, anxiety over expressed milk yield[24,25], and an increasing failure to meet mothers' own goals for human milk provision as time goes on[28].

As part of the rationale and preparatory work for this study, a Patient Public Involvement (PPI) survey of mothers of preterm infants was carried out to explore their experience of breastfeeding and expressing milk (appendix D). Among the 675 responses, mothers reported that the dominant problem they experienced with expressing was low milk volumes, and the most common question they had was how to express more milk. They also described the close relationship of stress and anxiety with milk volumes, which could operate in either direction – distress could lower their milk yield and low milk yield caused mothers distress. When asked to rank their breastfeeding related priorities, the top three were expressing more milk (32% of respondents ranked this first), breastfeeding for longer (25% ranked this first) and feeling that milk supply is secure (19% ranked this first). Mothers of the most preterm babies were more likely to say that expressing more milk was their highest priority.

5.4. Need for evidence

It is therefore of the utmost importance to gather high quality evidence on how to improve early expressed milk yield and long term exclusive HMF. The James Lind Alliance priority setting partnership identified 'What type of support is most effective at improving breastfeeding for premature babies?' in the top 10 priorities for research in preterm birth[29]. Despite this, a recent Cochrane review confirmed that there are few randomised controlled trials (RCTs) looking at human milk expression for premature babies[30]. Commercial interventional approaches such as modification of pumps and administration of oxytocin to the mother have predominantly failed to show a significant effect on outcomes, and the Cochrane review specifically recommended

that independently funded research is needed for trials on techniques that do not have commercial potential[30].

5.5. Guided relaxation interventions

One promising area is the use of simple guided relaxation and visualisation audio tracks to increase expressed milk yield, with two RCTs showing 60–200% increase in expressed milk yield after listening to these soundtracks, with a dose response relationship and increased effect in mothers of more unwell babies[31,32]. An increase in milk fat content was also seen. One further relevant paper reported a small RCT of mothers of preterm babies in a NICU setting listening to calming music while expressing milk during the first week of their baby's life. The increase in milk volume between day three and four of this study was doubled in the intervention group compared to the control group. The maternal anxiety score was also significantly lower in the intervention group[33].

Relaxation/visualisation tracks have also shown a decrease in maternal perceived stress and increased milk intake in healthy term babies at 6 weeks of age[34] and research is underway with healthy late preterm babies in the community[35,36]. The key studies are summarised in Table 1. One further RCT in breastfeeding mothers showed a reduction in physical parameters such as blood pressure and heart rate after listening to a relaxation meditation track as well as decreased perception of stress and some mothers experiencing MER while listening to the track, even though their infants were not present[37].

The theoretical basis of the effect of relaxation and visualisation on lactation is threefold – directly through stress hormones, directly through the central nervous system and indirectly through behavioural factors.

The production and release of milk are predominantly governed by the hormones prolactin and oxytocin. Close relationships have been noted between these lactogenic hormones and the wider web of pituitary hormones that affect perinatal mood[38]. Relaxation soundtracks have been shown to decrease maternal cortisol, which in excess can disturb the regulation of prolactin and oxytocin[27]. For example, salivary cortisol and a-amylase (which correlates with catecholamine levels and is another marker of stress) are associated with prolactin levels in mothers of very preterm babies over six weeks of expressing milk[39] and measures of anxiety such as the State-Trait Anxiety Index (STAI) correlate with oxytocin at 8 weeks after birth[40]. Oxytocin levels are also associated with serotonin levels and can rise in response to selective serotonin reuptake inhibitor (SSRI) treatment used to treat anxiety and depression[41]. There are therefore several plausible pathways whereby a relaxation intervention could modify the production and release of human milk hormonally.

Secondly, mental visualisation can utilise a central neuronal pathway to trigger MER (separate to the usual peripheral neuronal pathway stimulated by suckling or manual expression), which may be more effective when the visualisation is ritualised and repetitive due to operant conditioning. This is shown for example where mothers with high spinal cord injury can trigger MER with visualisation routines in the absence of any intact sensory pathways from the breast[42]. Operant conditioning has been noted to increase oxytocin levels before a baby starts to suckle or a pump is applied to the breast[39]. Mothers of preterm infants often report difficulties with MER due to the reduced physical contact and connection with their babies, and distress has

been causally linked to inhibition of MER. Inhibition of MER is particularly damaging as it leads to incomplete drainage of the breast and therefore secondary inhibitory feedback of milk production[41]. Therefore, the influence of the central nervous system and operant conditioning are plausible pathways for the effect of a stereotyped guided visualisation on lactation.

Thirdly there may be a behavioural mediator, whereby mothers who have less perception and manifestation of stress are more likely to interact positively with their infant[27], have increased self-efficacy[43] and therefore may 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.

Although the neonatal unit studies demonstrate the promise of the intervention, they looked only at short term outcomes and did not explore the mechanism of the effect, with only the smallest study measuring maternal mental health outcomes. Indeed, the first paper, published 30 years ago, recommended that looking at the effect of audio relaxation intervention on the long-term duration of breastfeeding was the logical next step[31], but this has still not taken place.

Table 1: Previous studies of audio relaxation interventions and human milk related outcomes.

	Feher et al, 1989	**Keith et al, 2012**	**Mohd Shukri et al, 2019**	**Varisoglu et al, 2020**
Study type	RCT	RCT	RCT	RCT
Patient Population	Urban USA, 71 mothers of "preterm" infants predicted to be in NICU for at least 10 days. Enrolled day 3–5	Urban USA, 162 mothers of NICU infants <38 weeks' PMA or critically ill. Most enrolled day 1–2	Urban Malaysia, 64 mothers of exclusively HMF term infants. Enrolled at 2 weeks of age	Urban Turkey, 44 first time mothers of infants 28–34 weeks' PMA
Intervention	20 min audio track of guided relaxation and visual imagery. Instructed to listen every day, especially prior to expressing, for 7–13 days	12 min audio track of guided relaxation and visual imagery. Either alone (D); or with lullabies (B); or with guitar music + images of the infant (C). Instructed to listen as often as possible while expressing for 14 days	Audio track of guided relaxation and visual imagery. Instructed to listen while breastfeeding, at least daily from 2 to 12 weeks of age	15 minutes listening to Turkish calming music during two expressing sessions on each of three consecutive days
Comparison group	No intervention	No intervention	No intervention (& unaware of nature of intervention)	No intervention

Patient characteristics (mean)	Gestational age: 30.8–31.5 weeks Maternal age: 24.5–26.8 years	Gestational age: 31.7–32.5 weeks Maternal age: 24.6–29.1 years	Maternal age: 59% were age 26–30 years and 33% were 20–25 years	Gestational age: 31.8–32.6 weeks Maternal age: 27.7–29.2 years
Primary outcome	Volume of single expression 4–13 days after enrolment	24-hour milk volume daily to day 14 after enrolment	Maternal stress & anxiety, breastmilk intake & infant anthropometry at 6 weeks of age	Increase in volume of average expression from 3rd to 4th day of study, maternal stress score on day 4 of study
Statistical test	t test	Repeated measures ANOVA	t test, ANOVA	Mann-Whitney
Results	Intervention milk volume: 90.1 ± 60 ml Control milk volume: 55.4 ± 48.2 ml $p < 0.05$	Intervention groups had higher milk volume from day 10 onwards. D14 mean yield Control group A: 318.2 ± 47.1ml Group B: 591.4 ± 47.6ml Group C: 1028.0 ± 48.8ml Group D: 861.7 ± 52.2ml $p < 0.0001$	At 6 weeks, intervention perceived stress score 12.6 ± 4.4. Control stress score 16.1 ± 5.9. p=0.01 Intervention group infants' mean adjusted milk intake was 227g/day higher than control group, p=0.03	Intervention STAI score: 40.1 ± 6.1 Control STAI score: 46.2 ± 3.5 p<0.005 Intervention increase in milk volume: 23.1 ± 14.1ml Control increase in milk volume: 12.3 ± 11.8 p=0.001
Notes	77% completed the study. 50% of women listened to the track >5 times before test expression.	Completion rate not reported. Frequency of listening not reported	97% completion rate. 80% intended to HMF for more than 19 months. Frequency of	Completion rate not reported. Publication is contradictory between written and

	Larger effect seen in mothers who listened more frequently and those whose babies were ventilated		listening not reported	graphical representation of results – clarified with the corresponding author
Criticism	Heterogeneous population with mixture of expressing and direct breastfeeding. Outcome difficult to interpret clinically. No outcomes related to mental health. Contamination not measured	Heterogeneous population with mixture of expressing and direct breastfeeding. Short term outcome. No analysis of mechanism of effect or maternal mental health. Contamination not measured	Very different study population. Some milk samples were lost in transport reducing the power to detect pre-specified outcomes	Small study, pre-specified primary outcomes not delineated, possibility of post-hoc fishing for significant result as increase in milk volume reported rather than absolute values. Contamination not measured

Although increasing early expressed milk yield is important in its own right, it is also necessary to see whether this intervention affects long term HMF outcomes. The first few weeks after birth have been proposed as a critical window to establish milk supply[13,44,45], so there is good reason to suspect such an impact. This RCT will analyse secondary outcomes at 36 weeks' PMA and 18 weeks after the EDD (approximately 4 months' corrected age).

5.6. Choice of outcomes

This RCT of a guided relaxation and visualisation audio track for mothers of very preterm babies will look at outcomes of expressed milk volume, longer term HMF and maternal mental health.

There is no recommended core outcome set for breastfeeding studies. PPI for this study (appendix D) dictated the choice of expressed milk volume as the primary outcome – mothers were very clear that low volumes of expressed milk are their major concern. Early milk expression volumes are an important outcome because low volumes of MOM will expose high risk infants to infant formula, or less immunologically active donor human milk depending to unit guideline, in the key timeframe of the first few weeks of life when mortality rate is highest. Maximum milk yield out of three measured timepoints in the first 3 weeks of life was chosen because some mothers of more mature babies will have started directly breastfeeding by day 21 so may have appropriately started to lower their expressed milk yield. It also allows mothers who terminate expressing before 21 days to be considered in the analysis.

Secondary outcomes were chosen after extensive PPI (appendix D) and consideration of the recommendation of Cochrane review to include outcomes related to maternal stress[30].

One set of secondary outcomes examine different facets of lactation at 21 days – efficiency of expression (weight of milk per minute of expressing time), which looks at the mechanism of any positive effect on lactation; and proportion of mothers achieving the Unicef BFI target for a secure milk supply (milk weight of at least 750g).

A second set of secondary outcomes assess HMF at different timepoints. Exclusive HMF is a well-established public health goal and 36 weeks' PMA is a commonly used timepoint for trial outcomes relating to neonatal admissions, as even very preterm babies with the smoothest admissions are unlikely to be discharged home before 36 weeks. Therefore 36 weeks' PMA provides a timepoint that is near discharge and also comparable between babies of different gestations at birth. A longer-term timepoint of 4 months' corrected age (18 weeks after the EDD) was chosen as all babies will be at least 6 months actual age at this point, so complementary feeds are indicated in most circumstances and this is the end of the expected exclusive HMF period and therefore the maximal demand on the mothers' milk supply. The timing of introduction of complementary feeds for preterm 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[46], making the definition of optimal length of exclusive HMF objectively difficult. However, it is important to examine the impact of the intervention at a timepoint near to the maximal demand on milk supply, as a suboptimal supply may be masked at earlier timepoints due to the limited volumes needed by a small baby.

A third set of secondary outcomes assess maternal mental health. Two standardised psychometric scales were chosen – the Spielberger State-Trait Anxiety Inventory (STAI) and the Post-traumatic stress Checklist (PCL-5). The STAI will be used in its short form to minimise burden on the mothers. The STAI is a reliable measure of anxiety[47] and widely used, including in studies of mothers after very preterm birth[5,48] and after use of calming music in the NICU[33]. The PCL-5 is a commonly used[49] screening assessment for post-traumatic stress disorder (PTSD) aligned to the Diagnostic and Statistical Manual of Mental Disorders-Fifth Edition (DSM-5)[50]. It has been found to be psychometrically sound[51–53] and has been used in the context of birth related trauma[54]. 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[5,55]. The PCL-5 was preferred by parent representatives over the IES because of more accessible language.

These two scales were chosen after consideration of a wide set of possible measures of maternal mental health, with the triple aims of being psychometrically sound, quick to administer and with wording appropriate to mothers of very preterm babies.

Process indicators were chosen to give more information on possible behavioural mediators of an effect on the primary outcome.

5.7. Exploratory analysis

Nested within the RCT is a second work programme to perform exploratory analysis of modifiable expressing factors, generating hypotheses for future work. Current expressing advice for mothers is extrapolated from the lactation physiology of mothers of term babies[45,56] rather

than from high quality evidence (see appendix C). Mothers find the Unicef Baby Friendly Initiative recommended expressing frequency (at least 8 times in 24 hours) and target yield (750–900ml by day 10–14) very challenging[25], and there is low adherence[30]. PPI for this study noted that mothers want to know many practical details about how to optimise HMF outcomes while minimising the demand on their physical and mental health – for example whether they can reduce the number of expressing sessions after a certain period of time and what expressed milk volumes they should target (appendix D).

The exploratory analysis will therefore look at the trajectories of expressed milk yield over time and whether these are altered both by the intervention and by other key factors such as expressing frequency and time to first expression after delivery. The relationship of maximal expressed milk yield and longer-term HMF outcomes will also be explored.

The exploratory analysis will use data collected for the RCT. Two extra study timepoints will contribute data for the exploratory analysis that are not required for the RCT – these are the expressing log at 32 weeks' PMA (only required for infants born at less than 27 weeks' PMA) and an assessment of HMF at 9 weeks after the EDD (approximately 2 months' corrected age). The extra log at 32 weeks assesses the maintenance of expressed milk yield for those mothers of extremely preterm babies, who must express for several months before the baby is developmentally ready to feed at the breast. This tackles the difficulty of including infants with a wide range of gestational ages at birth – for example the most preterm infant eligible would have a gap of 70 days between the expressing log at 21 days and the HMF assessment at 36 weeks' PMA, whereas the least preterm infant eligible would have a gap of only 8 days between these two points (see figure 2 & 3).

The extra HMF outcome at 2 months' corrected age assesses the trajectory of long-term feeding decisions, for example whether there is an early or late drop off for exclusive HMF after discharge. This will allow adjustment of the exploratory analysis if rate of exclusive HMF at 4 months' corrected age is too low to make any hypothesis of relationship between expressed yield and long term HMF. These two extra timepoints impose a small amount of extra time for the participants and research staff but make the dataset significantly more robust for exploratory analysis that will set future hypotheses and give information that will be useful for the design of future trials assessing these hypotheses.

5.8. Generalisation of results

Study outcomes will be broadly generalisable to extremely and very premature infants born in countries with advanced neonatal care, with the caveat of the limitations of any small trial. Inclusion criteria are broad to recruit a representative population, not just those who are highly motivated to express and exclusively breastfeed. The Oxford University Hospitals NHS Trust and Imperial College Healthcare NHS Trust neonatal units have a higher rate of HMF and exclusivity at discharge than the national average, but Manchester University NHS Trust has a lower rate than the national and regional averages[11]. All trusts have a dedicated infant feeding team and access to a dedicated psychologist or counsellor, but these are of variable size. The effect of the intervention may depend on the level of general lactation support so this range of contexts supports external validity. Further sites may be added if required.

There is likely to be some 'trial effect' – 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[57]. It is therefore likely that taking part in the study will to some extent modify mothers' expressing behaviours even in the control group. However, there is no way to overcome these limitations while robustly studying expression characteristics.

5.9. Benefits and risks

The benefit of this trial is that we will determine whether a simple, easily scaled up, non-commercial intervention improves expressed milk yield and exclusive HMF, which has the potential to benefit hundreds of thousands of preterm babies and their families globally. There are no major risks to participants. A minor risk to mothers participating in the trial is the potential for increased distress over poor lactation outcomes due to the focus on and measurement of these outcomes – this was highlighted in the PPI survey and mothers made suggestions to mitigate this through the language used in trial documents and by research staff. Many mothers also emphasised that this would be balanced by the positive effects of distraction and feeling that their experience was helping others.

The administration of standardised psychometric mental health scores as part of the trial could bring either benefit or risk to participants. If mothers score in a worrying range they will receive information about getting help, which may improve their psychological care. In contrast answering the questionnaires may make unacknowledged distress more obvious to mothers.

A smaller number of mothers within the PPI questionnaire mentioned the potential risk of the time burden of weighing milk and filling in questionnaires, which might take time away from their baby and other demands. The burden has been minimised by choosing three to four key time points for measurement rather than daily logs and keeping questionnaires short – estimated time to complete is 10–20 minutes at different timepoints. Mothers also advised keeping physical interference to a minimum by allowing participants to directly enter outcomes into an online platform. The longer term HMF outcomes are assessed by simple text message.

Since the initial design of the trial, the Covid-19 pandemic has occurred. This brings with it the risk that infection could be passed to participants by research staff. The minimal contact points of this trial are well suited to minimisation of transmission risk and procedures for electronic consent have been put in place. There will be pre-filled quarantined material packs for participants (weighing scale, cool bag, headphones) to eliminate risk of contamination. Research staff will wear appropriate personal protective equipment (PPE) and strictly observe recommended self-isolation periods in the event of symptoms or contact tracing.

6. OBJECTIVES AND OUTCOME MEASURES

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure

Primary objective To compare the expressed milk yield in intervention and comparator groups	24-hour weight of expressed milk on maternal log (highest from specified timepoints)	Day 4, day 14 and day 21 of baby's life
Secondary Objectives To compare the achievement of target expressed milk yield in intervention and comparator groups	Proportion expressing at least 750g milk in 24 hours, on maternal log on any of the specified timepoints	Day 4, day 14 and day 21 of baby's life
To compare expressing efficiency in intervention and comparator groups	Average volume of milk expressed per minute over 24 hours, on maternal log	Day 21
To compare maternal anxiety in intervention and comparator groups	Average maternal anxiety score (shortened STAI six-item self-report questionnaire)	Day 21
To compare maternal behavioural distress in intervention and comparator groups	Average maternal distress score (PCL-5)	Day 21
To compare exclusive human milk feeding in intervention and comparator groups	Proportion with exclusive human milk feeding by maternal report/from medical notes (no intake of infant formula in last 24 hours)	36 weeks' PMA; 18 weeks after EDD (4 months' corrected age)
To compare any human milk feeding in intervention and comparator groups	Proportion with any human milk feeding in last 24 hours by maternal report/from medical notes	36 weeks' PMA
Process indicators	Time spent in skin to skin contact with any of the mother's infants in 24 hours by maternal report (hours)	Day 21
	Number of expressing episodes in 24 hours by maternal report	Day 21
	Time spent expressing in 24 hours by maternal report (hours)	Day 21
Exploratory objectives Explore the trajectories of milk expression in intervention and control groups	Expressed milk yield in 24 hours; expressing frequency; time to first expression; treatment group	Day 4, day 14, day 21, 32 weeks' PMA (selected infants)

Explore the relationship of maximum expressed milk yield and exclusive HMF	Highest expressed milk yield in 24 hours from specified timepoints; exclusive HMF (defined as no use of infant formula in last 24 hours); treatment group	Day 4, day 14, day 21, 32 weeks' PMA (selected infants), 9 & 18 weeks after EDD (4 months' corrected age)

7. STUDY DESIGN

This is a non-blinded randomised controlled trial with parallel design taking place in three NHS trusts (further sites may be added if required). The entire recruitment and follow up period of the trial will take 22 months to complete and the trial aims to recruit 132 mothers of very preterm infants.

The recruitment period is divided into two sections – the 'long term follow up' recruitment period where participants will complete all outcome data points, and the 'main study only' recruitment period in the final 22 weeks (5 months) of recruitment where participants will end the study at OQ1 (36 weeks' PMA).

After recruitment and the baseline questionnaire (BQ), participants will fill out a process questionnaire and 24-hour expressing log, weighing all expressed milk at three time points until their infant/s are 21 days old (day 4, day 14 and day 21; PQ1-3). For babies born at less than 27 weeks' PMA there will be an extra time point at 32 weeks' PMA (PQ4). There is a simple two-question outcome measurement at 36 weeks' PMA (OQ1), which is the end of study for those recruited in the 'main study only' period. Participants recruited in the 'long term follow up' recruitment period will continue longer term follow up by completing the same outcome measurement (OQ2 & OQ3) at two further time points at 9 weeks after the EDD (approximately 2 months' corrected age) and 18 weeks after the EDD (approximately 4 months' corrected age).

Between day 4 and day 7, study staff will make contact with each participant to check that they understand the study processes, particularly how to weigh their milk.

Milk will be measured by weight due to its increased accuracy over visual assessment of volume. Simple online or text message based maternal questionnaires minimise time burden on the mothers.

The total duration of the RCT for each individual to the end of the 'main study only' period (36 weeks' PMA) varies depending on the gestational age at birth. Minimum duration is 29 days from the infant's birth. Maximum duration is 91 days from the infant's birth (see example timelines below). For those enrolled in the 'long term follow up' recruitment period there will be a further five months of follow up.

Figure 1: Trial Procedures

Procedures	

	From the day of birth (day 0) up to midnight on day 3 of life	**Day 4, 14**	**Day 4-7**	**Day 21**	**32 wk PMA**	**36 wk PMA**	**9 & 18 weeks after due date**
					If born <27 weeks only		*'Long term follow up' recruitment period only*
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						✓	✓

Figure 2: Sample timeline for most preterm infant eligible. Primary outcome timepoint highlighted in yellow, end of study for those recruited in the 'main study only' period highlighted in green

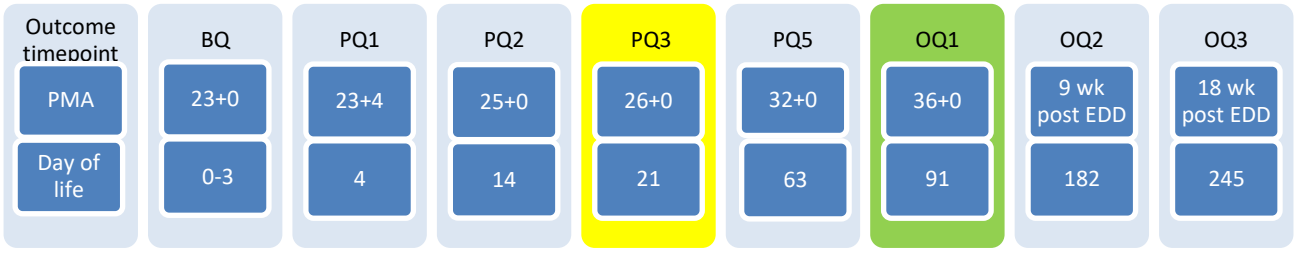


Figure 3: Sample timeline for least preterm infant eligible. Primary outcome timepoint highlighted in yellow, end of study for those recruited in the 'main study only' period highlighted in green

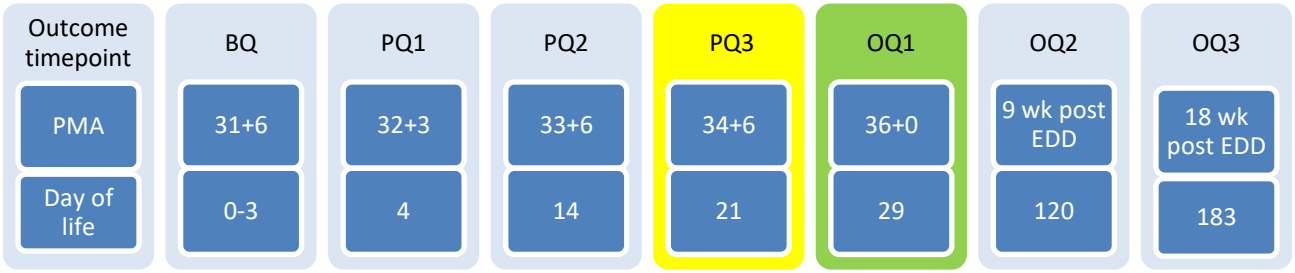
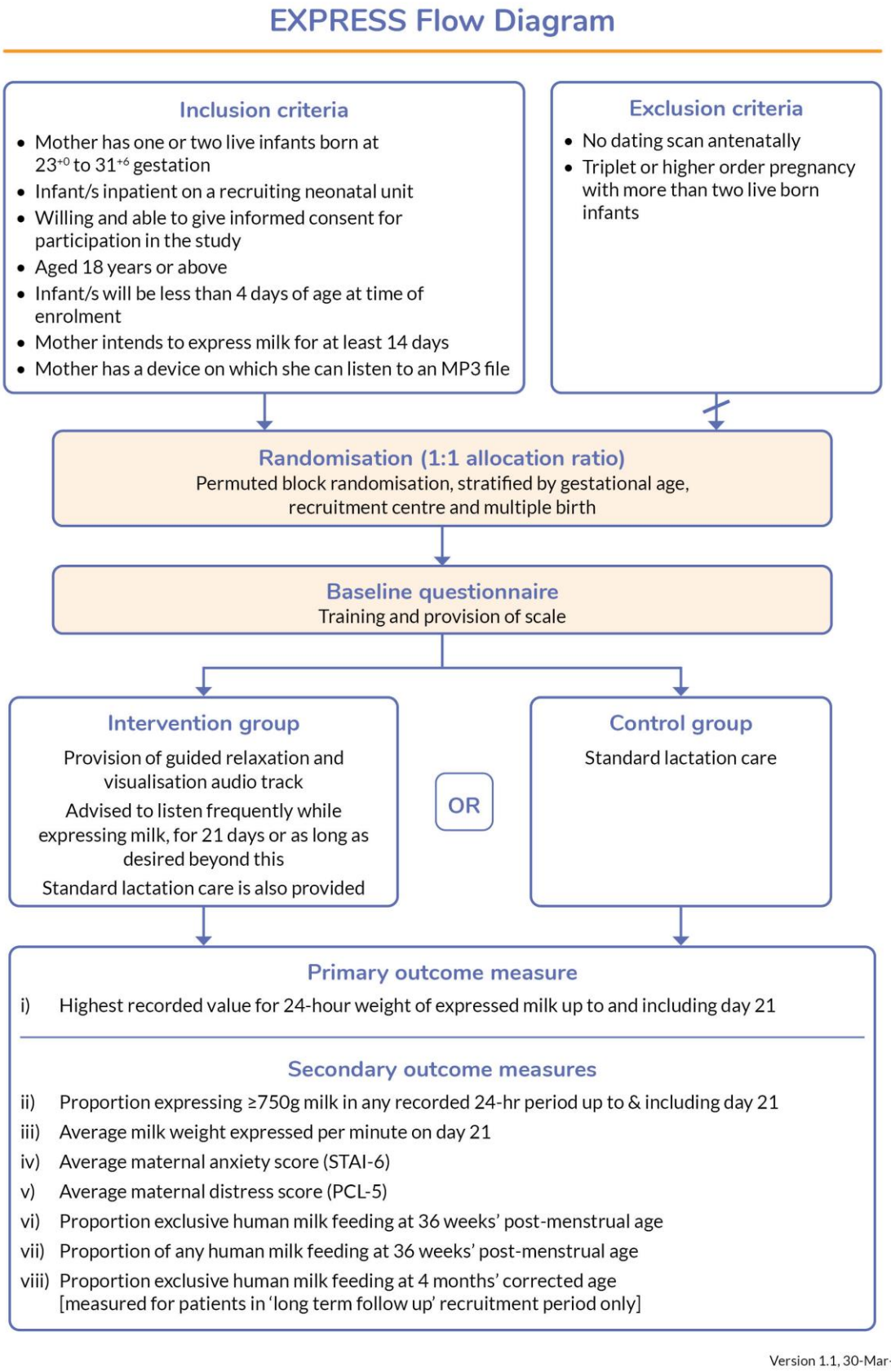


Figure 4: Trial flowchart



7.1. Risks related to study design

The use of a randomised controlled trial design minimises bias. It is not possible to blind the intervention to the mothers – any attempt at a placebo soundtrack risks either providing some relaxation or being actively anxiety-provoking, which would be unethical. The primary outcome is objectively measured making detection bias unlikely.

A risk related to lack of blinding is that mothers in the control group may also choose to listen to relaxing music or soundtracks because of their exposure to the trial literature or seeing mothers in the intervention groups, and/or that mothers in the intervention group are more motivated to remain in the trial leading to an imbalance between the groups in numbers completing follow-up. No realistic methods of eliminating these risks have been identified, but the contamination and proportion of missing data will be measured and reported, and the issue has been discussed with PPI representatives. The increased cost and complexity of a cluster randomised approach would not be justified in this setting. Community trials have used deception to bypass this risk (participants not aware of the exact nature of the intervention[34]), but this would not be practical in a neonatal unit where the intervention and control group participants have significant interaction. However, the design proposed for this study has been used successfully in a neonatal unit setting and the authors reported no contamination or increased drop out from control group participants, along with highly significant results (Keith & Weaver, personal communication).

8. PARTICIPANT IDENTIFICATION

8.1. Study Participants and feasibility

Participants are mothers of infants born between 23^{+0} and 31^{+6} weeks of gestation.

8.2. Inclusion Criteria

- Mother has one or two live infants born at 23^{+0} to 31^{+6} weeks of gestation
- Infant/s inpatient on a recruiting neonatal unit
- Willing and able to give informed consent for participation in the study
- Aged 18 years or above
- Infant/s will be less than 4 days of age at time of enrolment
- Mother intends to express milk for at least 14 days
- Mother has a device on which she can listen to an MP3 file

8.3. Exclusion Criteria

- No dating scan antenatally
- Triplet or higher order pregnancy with more than two live born infants

9. PROTOCOL PROCEDURES

See Figure 1 (summary schedule of procedures).

9.1. Recruitment

The primary recruitment centre will be the John Radcliffe Hospital neonatal unit, Oxford University Hospitals NHS Foundation Trust. The second recruitment centre will be Queen Charlotte's & Chelsea and St Mary's Hospital neonatal units, (this is a split site unit run by Imperial College Healthcare Trust). The third centre is St Mary's Hospital, Manchester neonatal unit. These centres all contain at least one tertiary neonatal unit and thus have the high concentration of very preterm infants required. Further sites may be added if required.

The clinical team will identify mothers who are eligible for inclusion in the study antenatally or shortly after birth and they will be informed about the study. There will be patient-facing posters on the antenatal and postnatal wards inviting those interested to contact staff or the research team. Mothers who are interested in the study will be given the opportunity to ask questions and will be given an information leaflet. Participants will then be screened and recruited by members of the research team by midnight of day 3 of life. The research team can discuss the trial with pregnant women at high risk of giving birth prematurely but they can only be screened and consented after birth so that the exact gestational age is known at randomisation.

The two recruitment periods – 'long term follow up' and 'main study only' will have slightly amended patient information leaflets with the correct description of study length and measurement timepoints.

9.2. Screening and Eligibility Assessment

Screening consists of assessment of inclusion and exclusion criteria.

9.3. Informed Consent

The participant must personally sign and date the latest approved version of the Informed Consent form before any study specific procedures are performed. Electronic consent form is available.

Because of the low risk nature of the trial, simple electronic consent (signature drawn with cursor or touch screen) with participant entered date will be used, as set out in HRA/MHRA guidance (2018). If a participant would like to consent electronically they provide their mobile phone number or email address to the researcher. They are then sent an electronic form with the same wording as the paper consent form. They are required to check the appropriate boxes, draw their signature and enter the date. Any attempt to change the date field would trigger an electronic alert to the central trial team and all attempted changes to forms are documented in an electronic audit trail. The researcher countersigns the consent form through their own login to the electronic database with a drawn signature and records the date. This process is hosted by OpenClinica, the same platform that hosts the electronic case report forms (eCRFs). The electronic consent form will be signed during a real time consent discussion, either in person, on the telephone or a virtual platform.

Written or electronic Participant Information Sheet (PIS) and written or electronic Informed Consent form will be presented to the participants detailing no less than: the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol,

and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal. Participants will have access to a member of the study staff, either in person, on the telephone or virtually, for further explanation and questions. A supporting podcast will be available but does not replace the provision of a written PIS.

The language used to describe the nature of the soundtrack in the patient information sheet has been kept to the minimum level needed for potential participants to decide whether to join the trial or not. If the soundtrack were described in detail it would be more likely that participants in the control group might be able to source a similar soundtrack. The language used to describe the soundtrack was suggested by the parent panel as striking the right balance between these two interests.

The participant will be allowed as much time as wished to consider the information, and the opportunity to ask questions to decide whether they will participate in the study. Written Informed Consent, which can be in electronic form, will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced and have been authorised to do so by the Chief Investigator or Principal Investigator. A copy of the signed Informed Consent will be given, or submitted electronically, to the participant. If written (rather than electronic) consent has been taken then the original signed form will be retained at the study site and a scanned copy will be sent securely to the NPEU CTU.

9.4. Randomisation, blinding and code-breaking

Participants will be randomised using stratified permuted block randomisation by a secure web-based randomisation program hosted by the NPEU CTU immediately after consent. Stratification will be based on centre, gestational age at birth (23^{+0} to 27^{+6} weeks versus 28^{+0} to 31^{+6} weeks) and multiple birth (one baby alive at time of randomisation versus two babies alive at time of randomisation). Telephone backup is available 24/7, 365 days a year. Code-breaking is not required.

9.5. Description of study intervention, comparator and study procedures (clinical)

9.5.1. Description of study intervention

The intervention is the provision of a specific audio track to the mother with a request to listen to the track during expression of milk as often as possible while expressing milk for at least three weeks. Participants will be sent a reminder text message on day 9 and day 17 to promote continued listening. The intervention audio track will last for approximately 12 minutes and consist of a guided relaxation and expression-specific visualisation – this is an adapted version of an existing soundtrack used for previous studies[32,34–36], modified and used under license 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 audio track will be provided as a downloadable mp3 file, with the option of an mp3 file on a USB stick if required. Mothers will be asked not to share the file with anyone else during the study. They can continue listening to the track throughout the study period and beyond if they so desire.

In addition, the intervention group will receive standard care, including lactation advice from neonatal unit staff and infant feeding team and standardised printed information describing best-practice information on how to express milk for a preterm infant[58].

9.5.2. Description of comparator

The comparator group will receive standard care, including lactation advice from neonatal unit staff and infant feeding team and standardised printed information describing best-practice information on how to express milk for a preterm infant[58]. It is standard care to encourage skin to skin contact between mother and infant/s for many reasons, including because of its positive impact on milk supply and establishment of breastfeeding.

9.5.3. Description of study procedures

Mothers will be given a portable accurate electronic scale at recruitment to the RCT and trained on its use. Mothers will enter data directly into electronic case report forms with automated reminders. They will also have the option of printed forms to return with Freepost envelopes to the CTU or to upload securely by taking a photograph of the forms. The final outcome data will use answers to text messages.

Participants can express milk by whatever method they desire. The recommended method is use of a double hospital-grade electric pump. These are provided on the neonatal unit along with information on hire for home. On measurement days mothers will weigh all milk they express while noting the time of expression and length of each expression session. Single use scales accurate to 0.1g, which have been tested before use, will be provided to mothers to weigh the milk inside a collection bottle/syringe/cup, with the lid on. Standard container weights are known – if participants use an unknown container they will be asked to weigh the empty container as well as the container with milk in it. Participants will be given instruction on how to operate the scale at randomisation. They will be asked to demonstrate use of the scale and their competence will be recorded.

9.6. Baseline Assessments

Participants will fill out a baseline questionnaire at recruitment (BQ) either in paper form or electronically in OpenClinica according to their preference. Electronic forms can be completed on the mother's smartphone or other device, or on a study tablet. Paper forms will be returned to research staff and stored in the NPEU after input into OpenClinica. Research staff also fill out a Trial Entry Form immediately after randomisation.

Baseline information will be collected on sociodemographic and other characteristics, including:

- Type of pregnancy, mode and location of birth
- Assessment of how unwell both mother and baby/babies are at randomisation
- Previous breastfeeding experience and breastfeeding intention
- Time to first expression of breastmilk
- Maternal postcode, education level, ethnic origin and smoking status
- Baseline STAI-6 score

9.7. Subsequent Assessments

Measurements are inputted directly by mothers into OpenClinica via a link sent to them at each timepoint – data can be entered throughout the day or at the end of the 24-hour period. Text message reminders will be sent 12 hours prior to each process measurement day, and further email reminder on the morning of the measurement day, both automated. Telephone/email/SMS contact will be made within the acceptable deviation period if the record is not submitted. Paper forms and expressing logs will be available if preferred, with Freepost envelopes or an option to upload photographs of paper forms. Electronic forms can be completed on the mother's smartphone or other device, or on a study tablet if the mother remains in a recruiting centre.

9.7.1: Process measurements PQ1-3 (universal) and additional PQ4 (according to gestational age)

For each of the process measurement days, participants are instructed to start the 24-hour record at the time they first express milk after waking up in the morning and continue for a period of 24 hours. They will also receive a questionnaire collecting data on:

- Number of direct breastfeeds (if any), dose of skin to skin, perception of milk supply
- How often they have listened to the audio track and how they feel about it (intervention group only, measuring compliance and attitudes to intervention)

- **Day 4 after birth (PQ1)**
  - Participant fills in questionnaire
  - Participant records the time/length/nature of each episode of milk expression and the weight of the resulting milk, for a period of 24 hours
  - Acceptable deviation for the start of the expressing record is 48 hours

- **Day 14 after birth (PQ2)**
  - Identical procedure and acceptable deviation period to PQ1

- **Day 21 after birth (PQ3)**
  - Questionnaire has additional mental health scoring questionnaires (STAI-6 and PCL-5) and questions on whether they have listened to or practice independently sourced relaxation material (to measure contamination).
  - Identical procedure and acceptable deviation period to PQ1

- **32 weeks' PMA (PQ4)**
  - This measurement is only required for mothers who gave birth at <27 completed weeks of gestation
  - Identical procedure to PQ1
  - Acceptable deviation for the start of the expressing record is 96 hours

9.7.2: Outcome measurements OQ1-3

- **36 weeks' PMA (OQ1)**
    - Participant responds to a text message asking whether the baby has had maternal breastmilk and infant formula in the last 24 hours
    - Acceptable deviation period is 7 days
    - Infant feeding status can be extracted from infant's medical notes if no text message response is received
    - OQ1 is the final measurement for mothers enrolled in the 'main study only' section of the recruitment period

- **9 weeks after EDD (OQ2)**
    - Identical procedure and acceptable deviation period to OQ1

- **18 weeks after EDD (OQ3)**
    - Identical procedure and acceptable deviation period to OQ1
    - OQ3 is the final measurement for mothers enrolled in the 'long term follow up' section of the recruitment period

9.8. Withdrawal of Participants from Study

Each participant has the right to withdraw from the study at any 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 during the study period

The number of participants withdrawn will be reported by trial arm. Participants who are withdrawn will not be replaced. The reason for withdrawal will be recorded in the CRF for that participant.

Participants choosing to stop treatment (listening to the intervention soundtrack) but continuing follow up will remain in the study.

9.9. Definition of End of Study

The end of study is defined as database lock.

10. SAFETY REPORTING

No adverse events, including serious adverse events, will be reported due to the established and inherently low risk nature of the intervention.

11. STATISTICS AND ANALYSIS

11.1. Statistical Analysis Plan (SAP)

The statistical aspects of the study are summarised here with details fully described in a statistical analysis plan (SAP) that will be available prior to database lock.

11.2. Description of Statistical Methods

Section 6 summarises outcome measurement variables and time points for analysis. Demographic and clinical data will be summarised with counts and percentages for categorical variables, means (with standard deviations) for normally distributed continuous variables and medians (with interquartile or simple ranges) for other continuous variables.

Continuous outcomes will be analysed using linear regression with 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 for analyses of the primary, secondary and process outcomes.

Analyses will be adjusted for the stratification factors (centre, gestational age at birth and whether the participant has one or two babies alive at randomisation) where possible. The primary outcome and relevant secondary outcomes will be adjusted for the day of milk yield contributing. The STAI-6 score at day 21 will be adjusted for the STAI-6 score at baseline. 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, with no comparative statistics presented. Sensitivity analyses will take place.

Exploratory analysis will use generalised linear modelling, correlation analysis, ROC curves and survival analysis.

Due to the nature of this clinical trial as a component of the Chief Investigator's PhD, the CI will carry out the statistical analysis after database lock. Prior to database lock the CI will prepare statistical programs with dummy data to remain blinded to aggregate data while interacting with participants. The trial statistician will run the programs to produce relevant reports for the Project Management Group and Data Monitoring Committee while the trial is ongoing, as outlined in the trial Risk Assessment.

11.3. Sample Size Determination

Previous work has shown increase in yield with audio relaxation intervention of between 60% and 270%[31,32]. In the most relevant study[32], mean yield at day 14 increased from 318ml ± 309ml to 862ml ± 309ml with the use of a visualisation/relaxation soundtrack. Exploratory work in the Thames Valley showed that baseline milk yield is higher and more variable than seen in that trial, although the standard deviation is likely to be overestimated due to small sample size (table 2). The study is therefore powered to detect an increase in maximum expressed milk yield from 670g ± 300g to 825g ± 300g (23% increase), with 80% power and a two-sided significance level of 0.05. 118 participants are required at day 21. Note that human milk has an average specific gravity of 1.03[59,60] therefore 670g is estimated as equivalent to 650ml and 825g is estimated as equivalent to 800ml.

Table 2: Exploratory work used for power calculation

	Nature	**Time period**	**Number**	**Characteristics (mean)**	**Useful results**
First phase	Small clinical audit, convenience sample of preterm babies (maternal interview)	2017–2018	32 mothers, 41 babies	GA 29+1 BW 1312g	**Mean yield ± standard deviation (median)** Day 14: 628 ± 465ml (510ml) Day 30: 723 ± 434ml (600ml) **Exclusive HMF (defined as protocol)** Discharge: 42% 4wk post term: 21% 14wk post term: 13%
Second phase	Large clinical audit, complete sample of all single/twin babies born <32 week or transferred in within 48 hours of birth (prospectively collected)	2016–2018	196 mothers, 269 babies	GA 28+0 BW 1056g	**Mothers whose infant/s died before 21 days** 10.7% **Babies ever received MOM** 90.1% **Definitely or probably receiving MOM day 21** 81% **Exclusive HMF** Discharge home: 38%

To achieve 118 participants remaining at 21 days, the recruitment target has been set at 132, to allow for a 10% mortality rate. Recruitment targets are 100 at the Oxford University Hospitals NHS Trust and 32 at Imperial College Healthcare NHS Trust.

Approximately 180 babies born at less than 32 weeks' PMA are cared for in the Oxford University Hospitals NHS Trust neonatal unit in the first few days of their life each year. Because of multiple births, the pool of eligible mothers is approximately 130 per year. Approximately 120 babies born at less than 32 weeks' PMA are cared for in the Imperial College Healthcare NHS Trust neonatal units in the first few days of their life each year, giving an expected pool of eligible mothers of 90 per year.

Recruitment is planned to start in the John Radcliffe Hospital in April 2021 for a period of 19 months – the final 22 weeks of this period will be 'main study only' recruitment, the rest will be 'long term follow up' recruitment. The recruitment target is 49% of eligible mothers at the John Radcliffe Hospital.

Recruitment is planned to start in the Imperial College Healthcare NHS Trust in October 2021 for a period of 13 months – the final 22 weeks of this period will be 'main study only' recruitment, the rest will be 'long term follow up' recruitment. The recruitment target is 33% of eligible mothers at Imperial College Healthcare NHS Trust. Given the lack of risk for the infant, a recruitment target of less than 50% is realistic and achievable. Further sites may be added if required.

It is estimated that 93 participants will be recruited in the 'long term follow up' period, and 39 in the 'main study only' period. Allowing for 10% mortality and 20% drop out by the final outcome timepoint of 18 weeks post EDD, it is estimated that 66 participants will remain at the end of the long term follow up period.

11.4. Outcome measure definitions

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 mother has two babies, the outcome will be classified with reference to both infants (any HMF given to either infant; any infant formula given to either infant). Of note, intake of complementary food is not considered in this outcome.

Maternal anxiety will be measured by the STAI-6. The original Spielberger STAI is a 20-item questionnaire with four answer options for each question ('not at all', 'somewhat', 'moderately' or 'very much') and a score of 1–4 for each question. The total score range is 20–80, with higher scores signifying more anxiety, <36 generally considered normal[61] and ³40 signifying clinically significant anxiety[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[62]. The short form is highly correlated with the 20-item STAI, with internal consistency greater than 0.9[63]. The minimal meaningful difference in STAI score has been suggested as 10[64].

Maternal stress reaction to the trauma of preterm birth will be measured by the PCL-5. By definition PTSD cannot be diagnosed at 21 days after the traumatic event but this score will measure post-traumatic stress reactions, which are very prevalent after preterm birth[5] and highly salient and important to mothers in PPI panels for this trial. Although relaxation techniques are not a standard treatment for PTSD, they have been shown to modify the autonomic response to stress in PTSD[65]. The visualisation aspect of the soundtrack could also theoretically increase connection to the infant, which is a component of an effective intervention shown to reduce PTSD in mothers of preterm infants[66].

The PCL-5[50] has 20 items with four answer options for each question ("not at all", "a little bit", "moderately", "quite a bit" and "extremely") and a score of 0–4 for each question. The total score range is 0–80, with higher scores signifying more distress. A cut off of 31–33[5152] has been proposed as indicative for probable PTSD. A minimal meaningful impact of treatment has been suggested as bringing the total score down below 24[51], although this should be used with caution. An alternative approach is that a 5–10 point change is likely to be the minimum threshold for a clinically meaningful difference[50]. The PCL-5 has internal consistency (for example Cronbach's alpha 0.95) and good construct validity in a variety of settings[51–53] although of necessity the evidence is limited because of its recent modification to align with DSM-5 criteria.

Process indicators of time spent in skin to skin (number of hours; sum of all hours if twins), frequency of expressing (where two breasts are expressed either simultaneously or if the start time of the second breast expression is within 10 minutes of the end time of the first breast expression, this is defined as one episode of expressing) and time spent expressing (number of hours) will also be analysed in relation to the intervention.

11.5. Analysis Populations

Mothers will be analysed in the groups to which they were randomly assigned, comparing the outcome of all infants allocated to intervention with all those allocated to the comparator group, regardless of deviation from the protocol or treatment received (referred to as the Intention to Treat (ITT) population).

Exploratory subgroup analysis will use the statistical test of interaction to examine the effect of the intervention on the primary outcome by gestational age (<28 weeks' PMA at birth and ≥ 28 weeks' PMA at birth). Stratified analysis by the other stratification factors described and exclusive HMF intention at baseline will take place with no formal test for interaction.

An exploration of compliance effect on primary outcome will be conducted, dividing the intervention group into high and low frequency (listening to the intervention soundtrack 2 times a day or more, or fewer than 2 times a day). 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).

Basic demographics of the recruited participants will be compared with anonymous data from the eligible population (gestational age at birth, maternal age, multiple pregnancy, delivery mode) to assess external validity.

11.6. Exploratory Analysis

There will be two objectives used for exploratory analysis. Firstly, the relationship of highest expressed milk yield at a 24-hour log timepoint with exclusive HMF at 9 weeks and 18 weeks after the EDD (approximately 2 and 4 months' corrected age). Effect of maternal HMF intentions and other potential confounders will be adjusted for. The analysis will aim to identify a volume that is most strongly associated with long term outcomes, and a time point for this target.

Secondly, the repeated measures of expressed milk yield at all process time points will undergo exploratory analysis to look at the trajectories of yield and relationship of intervention group, time to first expression and expressing frequency. This analysis will use the target yield/timepoints identified in the first part as an outcome, or 750g yield by day 21 if none has been identified[56]. The first pre-defined hypothesis is that there is an interaction between expressing within 2 hours of delivery and the frequency required to gain and maintain adequate yield. The second pre-defined hypothesis is that after target milk yield is achieved, expressing frequency no longer correlates with yield.

11.7. Decision points

There will be no formal interim analysis or decision points due to the size of the trial. The DMC will view populated statistical dummy tables to monitor safety, statistical assumptions and data completeness (without statistical tests being performed).

11.8. Stopping rules

No formal stopping rules required.

11.9. The Level of Statistical Significance

Two-sided significance level of 5%.

11.10. Procedure for Accounting for Missing, Unused, and Spurious Data.

The primary outcome uses the highest value for 24-hour milk weight out of the three measured time points up to and including day 21, regardless of missing values. Sensitivity analyses will use imputation of missing values, and will assess the impact of using only day 21 24-hour milk weight (to account for bias in the pattern of missingness between days).

11.11. Procedures for Reporting any Deviation from the Original Statistical Plan

All deviations from the original statistical analysis plan will be reported in the final report, as appropriate.

12. DATA MANAGEMENT

The data management aspects of the study are summarised here with details fully described in the Data Management Plan.

12.1. Source Data

CRF online entries by participants or their paper equivalent are the main source documents in this study. A researcher will extract necessary data for randomisation and baseline questionnaire from the infant and mother's medical records at recruitment only. Text message responses by participants will be entered into the CRF automatically.

Maternal postcode will be transformed to Index of Multiple Deprivation decile/quintile using national reference databases.

Basic demographics of the eligible population (gestational age at birth, maternal age, multiple pregnancy, delivery mode) will be extracted from routinely entered clinical data from the BadgerNet platform by clinical staff and fully anonymised before input into OpenClinica screening logs.

12.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations.

12.3. Data Recording and Record Keeping

Data will be entered directly into an online CRF (case report form), either by the participant or a health professional tasked with that role on the delegation logs at sites, or by members of the study team, or automatically through the response of a participant to a study text message. The online CRF is part of the clinical database management application OpenClinica, used by the NPEU CTU to undertake the management, storing and curation of clinical data. Access to OpenClinica is controlled by the CTU according to role and training.

The OpenClinica application is hosted in the UK by an ISO 27001:2013 (Information Security) accredited third party (AA).

An option to upload photographs of paper CRFs will be available to participants. Photographs will include the study ID number but no directly identifiable information. Files will be uploaded by participants via a URL to an instance of the electronic survey provider LimeSurvey that is hosted on the secure servers of the NPEU. Files will be encrypted during transfer.

All administrative data collected for the study (including patient contact details) will be entered into the study version of the NPEU CTU trial administration database application (TADA). TADA is a web-based application hosted on secure restricted servers by the NDPH. Access to TADA is restricted to members of the study team and the Trials Programmers, who have access to the servers and underlying database. TADA will generate automatic invitations to participants to use unique web links to fill in data at specified timepoints..

Electronic documents related to the study are stored in a directory on a secure network hosted by the Nuffield Department of Population Health (NDPH), University of Oxford, with controlled access according to role.

At the end of the study the two databases (patient identifiable details, and separate anonymised main dataset) will be stored on secure university servers within the NPEU (located in the NDPH), which are backed up daily. Backups will only be stored within the University backup system and only accessible via fully authenticated, traceable means. Version control will be enforced for the data and for Stata ‘do’ files used in the analysis, new iterations of the dataset will be saved with a clear filename and time stamp. After the trial has been completed and the reports, journal articles and thesis published, the data will be archived in a secure electronic location with controlled access.

All data will be processed in line with the NPEU CTU Data Management Standard Operating Procedures (SOPs). The Sponsor has delegated the responsibility for ensuring confidentiality of participant information to the NPEU CTU. All paper and electronic data will be stored securely in strict compliance with data protection regulations.

No transfer of personal data will take place during the study. If data transfer is requested by external researchers in future years this will take place via an appropriately secure communications procedure authorised by the University of Oxford. A review of all requests for sharing of the study data will take place and will be managed in accordance with NDPH policies.

13. QUALITY ASSURANCE PROCEDURES

The study may be monitored, or audited in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures.

13.1. Risk assessment

A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the study to reflect significant changes to the protocol or outcomes of monitoring activities.

13.2. Study monitoring

Regular monitoring will be performed according to the study specific Monitoring Plan.

13.3. Study Committees

13.3.1. Project Management Group

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 PMG will consist of the Chief Investigator, CTU Director, Senior Trials Manager, Core Trials Support Role, Trial Programmers, the Trial Statistician, the Quality Assurance manager and Trial Administrator. The PMG will meet every three to four weeks.

13.3.2. Data Monitoring Committee

The Data Monitoring Committee will meet twice times during the 36-month period of the doctorate. It will consist of three independent statistical and clinical experts and will interrogate the data coming out of the trial to ensure there are no impediments to continuation.

13.3.3. Trial Steering Committee

The Trial Steering Committee will meet three times during the 36-month period of the doctorate. It will consist of independent clinical and academic experts, senior CTU staff, the Chief Investigator, a Bliss representative and a veteran parent – this is the executive body for the trial, which will make decisions in light of the data monitoring committee recommendations, for example relating to safety or futility, and ensures that the trial is conducted according to the principles of GCP. Meetings will be conducted according to the NPEU SOP for the composition and role of trial committees.

14. PROTOCOL DEVIATIONS

A study related deviation is a departure from the ethically approved study protocol or other study document or process (e.g. consent process or administration of study intervention) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations

from the protocol will be documented in a protocol deviation form and filed in the study master file.

15. SERIOUS BREACHES

A "serious breach" is a breach of the protocol or of the conditions or principles of Good Clinical Practice which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the trial subjects; or

(b) the scientific value of the research.

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the approving REC committee and the relevant NHS host organisation within seven calendar days.

16. ETHICAL AND REGULATORY CONSIDERATIONS

16.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

16.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

16.3. Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), and HRA for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

16.4. General Ethical Considerations

The trial will run in the context of families experiencing the trauma of very preterm 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 will 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. We have worked with parent panels and Bliss to ensure that our trial structure and the language of all of our trial documents takes this situation into account. 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 exceed a pre-specified threshold for concern, the participant will be sent information on self-help and information about how to access NICU based psychological support.

16.5. Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA (where required), host organisation and Sponsor. In addition, an End of Study notification and final report will be submitted to the same parties.

16.6. Transparency in Research

Prior to the recruitment of the first participant, the trial will have been registered on a publicly accessible database. The trial information will be kept up to date during the trial, and the CI or their delegate will upload results to the public registry within 12 months of the end of the trial declaration.

16.7. Participant Confidentiality

The study will comply with the General Data Protection Regulation (UK GDPR) and Data Protection Act 2018, which require data to be de-identified as soon as it is practical to do so. The processing of the personal data of participants will be minimised by making use of a unique participant study number only on all study documents and any electronic database(s). All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data.

16.8. Expenses and Benefits

Each participant will be provided with a small digital weighing scale, a coolbag and a pen. They will be offered a set of headphones and a USB stick if in the intervention group. They can keep any of these pieces of equipment at the end of the study. The collective value of these items is less than £15.

All participants will be emailed an electronic food and drink voucher code for £10 with the link for their day 14 or day 21 expressing log. 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.

17. FINANCE AND INSURANCE

17.1. Funding

The study is funded by the National Institute for Health Research (NIHR), via a Clinical Doctoral Fellowship award.

17.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London).

17.3. Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties.

18. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by the National Institute of Health Research (NIHR). Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

A results summary will be disseminated to study participants within 18 months of the End of Study, if they provided contact details to do so at the time of consent.

19. DEVELOPMENT OF A NEW PRODUCT/ PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

Ownership of IP generated by employees of the University vests in the University. The University will ensure appropriate arrangements are in place as regards any new IP arising from the trial.

20. ARCHIVING

On completion of the datasets and following publication of the study results, datasets will be encrypted and archived in a secure electronic archive. Future access will be controlled by the data custodian (Director of the NPEU; currently Prof Jennifer Kurinczuk) and would be subject to further regulatory approvals. When the data are no longer required, the destruction and disposal of data will be performed in accordance with good practice. It is NPEU policy for clinical trials to keep patient contact information for at least 25 years after the study has finished for the unlikely event that a very long-term effect of the treatment is discovered or needs further research – however because the nature of the trial intervention makes this very unlikely the data will be reviewed five years after the completion of trial activity to assess whether there is continued justification for data retention.

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22. APPENDIX B: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	2.0	22/04/2021	Ilana Levene	Inclusion of a day 4-7 check in contact with an aim to increase accuracy of expressing logs. Explicit mention of access to both mother and baby's medical notes for Trial Entry Form. Minor amendments to CRF contents (Apgar score, type of expressing, change in assessment of contamination). Clarification of nature of e-consent signature. Details added of data blinding, ethical considerations. Reduction of subgroup analysis planned. Clarification that Imperial has two neonatal unit sites.
2	3.0	26/11/2021	Ilana Levene	Inclusion of a £10 thank you gift in the first three weeks after recruitment. Clarification of the action if mental health scores exceed a clinical cut off. Amendment of details of the text message CRFs, which will be a single text message rather than two for simplicity. Amendment of the exact details of electronic consent dating. Update to name of university sponsor office from CTRG to RGEA.
3	4.0	30/03/2022	Ilana Levene	Inclusion of a process for participants to upload photographs of paper CRFs References to the nature of the third recruitment site Updates to statistical analysis plan
4	5.0	26/05/2022	Ilana Levene	Noted that infant feeding status at 36 weeks' PMA can be derived from infant medical notes if no participant SMS received

List details of all protocol amendments here whenever a new version of the protocol is produced. This is not necessary prior to initial REC submission.

23. APPENDIX C: Summary of evidence on modifiable expressing parameters and HMF outcomes

Note, systematic review of different types of pump and of simultaneous versus sequential pumping were felt to be sufficient (Becker 2016) so they are not included in this evidence review.

Search terms: (((breastfe* OR (human NEAR milk) OR (breast NEAR milk) OR (mother* NEAR milk))) AND (preterm OR prematur* OR vlbw OR elbw)) AND (express* OR pump*) à 349 records in March 2019. 82 abstracts were reviewed and 30 full text reports obtained. 13 additional full text reports were reviewed from hand searching reference lists, citation searching and personal communication. The search was updated in July 2020 and 3 further studies included. Some studies stimulated several different reports. 29 studies were therefore included in total in the evidence review and are described below:

Time to first expression of milk

Paper	Study type	Finding
Parker 2019b	Very large cohort	Identified expressing **within 8 hours** of birth as the cut-point best associated with any and exclusive MOM at discharge home/transfer to lower care level unit. Adjusted hazard ratio of any MOM at discharge/transfer was 0.45 for expression 9–24 hours after birth, compared to within 8 hours of birth
Parker 2019a	Very large cohort	Adjusted odds ratio of any HMF at discharge was 1.5 for expressing **within 6 hours** of birth
Levene 2019	Small audit	Expressing **within 2 hours** of birth associated with increased yield at mean 30 days of age, from median 600ml to 814ml (p=0.04) Expressing **within 2 hours** of birth associated with increased achievement of yield ³750ml at mean 30 days of age, from 25% to 76% (p=0.03)
Parker 2017 & Parker 2020	Medium size RCT	First expression **1–3 hours** after delivery increased any HMF at discharge from 35% to 62%, compared to 3–6 hours. This study was compromised by significant differences in the characteristics of the different arms and a high frequency of expression sessions with zero milk yield and is therefore difficult to interpret
Maruyama 2016	Medium size cohort	Adjusted odds ratio of exclusive formula feeding at discharge was 1.06 for **each hour** of delay to first expression (p=0.002)
Berrani 2015	Medium size cohort	Odds ratio of any HMF at 6 months was 5.1 for first expression **within 48 hours** of birth, compared to 3 days or more (p=0.001)
Maastrup 2014	Large cohort	Odds ratio of non-exclusive HMF at discharge was 4.9 for expressing **more than 48 hours** after birth, compared to expressing within 6 hours of birth (p<0.01)

		Odds ratio of non-exclusive HMF for the nationally recommended duration was 1.6 for expressing 12–24 hours after birth, compared to expressing **within 6 hours** of birth (p<0.05)
Murphy 2014	Small cohort	Expressing **within 6 hours** of birth associated with increased exclusive MOM at 28 days, from 44% to 93% (p=0.008)
Parker 2012	Small RCT	First expression **within 1 hour** of birth increased volume of expressed milk at week 3 from 267ml/day to 613ml/day, compared to expression 1–6 hours after birth (p=0.01)
Hill 2015	Medium size cohort	Time of initiation of breast stimulation after birth was significantly inversely correlated with early milk output (week 1: r = 0.21, p = .04) but not correlated at weeks 2 to 6
Furman 2002	Medium size cohort	Any HMF at 4 months corrected age associated with higher rate of expressing **within 6 hours** of birth from 9% to 30%, compared to no HMF at 4 months corrected age (p=0.02)
Hill 1999 & 2001	Secondary analysis of small RCT	Time to first expression associated with average milk weight from week 2–5 (p=0.04, Spearmans rho -0.3). In subgroup analysis this was significant only for the 'low expressing frequency' group (mean 5/day), not the 'high expressing frequency' group (mean 7/day)

Expressing frequency

Paper	Study type	Finding
Ru 2020	Small cohort	Expressing ≥**6 times/day** associated with higher yield at **42 days**, from average 558ml to 1179ml (p=0.009). Non-significant trend for the same relationship on day 7 and 14 (300ml to 490ml; 448ml to 769ml respectively). Average yield was 725ml by day 14
Lai 2019	Very small cohort (repeated measures)	Daily yield at **day 10–20** was not significantly different between those expressing **5, 6, 7, 8 and 9 times/day**. Those expressing 6 times/day had an average daily yield of 228g more than those expressing 3 times/day (p=0.006). Modelling daily yield from number of daily pumping sessions gave a prediction of 732ml at 5 times/day, 784ml at 6 times/day and 805ml at 8 times/day
Patel 2019	Large cohort	In multivariate logistic regression, mean number of daily pumping sessions during the first 14 days predicts any HMF at discharge (odds ratio 1.71, p = 0.001)
Hoban 2018b	Very small cohort	Mothers achieving target yield (³500ml/day by **day 14**) expressed **median 6.5 times/day** whereas mothers who didn't achieve target yield expressed median 4 times/day (p<0.001) 86% of mothers who expressed **³5 times/day** achieved target yield, whereas 22% of mothers who expressed <5 times/day did so (p= 0.01).

Levene 2019	Small audit	Expressing frequency correlated with yield on day 7 and day 14 but not at 3 weeks or later. Expressing **>6 times/day** associated with higher yield at **14 days**, from median 348ml to 926ml (p=0.003)
Fewtrell 2016	Secondary analysis of small RCT	Expressing frequency in **first 10 days** not associated with mean yield/day throughout admission
Lussier 2015	Secondary analysis of Small RCT	Daily milk volume over the **first 28 days** associated with the number of expression sessions per day, with **each session** adding an adjusted 25mL to daily volume (p<0.001)
Bishara 2009	Small cohort	Among mothers producing at least 120% of their infant's enteral requirement, expressing frequency **around week 3** not associated with milk volume
Morton 2009	Small cohort	Hand expressing **>5 times/day** on day 1–3 (in addition to pumping mean 5 times/day) associated with higher yield on **day 14** than hand expressing <2 times/day, from median 443ml to 780ml (p=0.001) Electric pumping frequency associated with mean yield during week 2 (p=0.008) and at week 8 (p=0.002) Expressing **³7 times/day** associated with higher yield at **2 weeks**, from median 402ml to 622ml (p=0.03), but not at **8 weeks**, median 752ml and 1019ml (p=0.2)
Furman 2002	Medium size cohort	Any HMF at 4 months corrected age associated with higher rate of expressing **³5 times/day** at **week 3**, from 63% to 87%, compared to no HMF at 4 months corrected age (p=0.04)
Hill 1999	Secondary analysis of small RCT	Average frequency of expressing in **week 2–5** associated with average milk weight (p=0.005, Spearman's rho 0.44)
De Carvalho 1985	Small RCT	Expressing **³4 times/day** increased expressed milk yield at **day 11–18**, from 221ml to 342ml

Milk yield in first 3 weeks of life

Paper	**Study type**	**Finding**
Ru 2020	Small cohort	The 24-hour yield on day 7 that maximises sensitivity and specificity to predict target yield **(³750ml by day 42**) is 407ml. The 24-hour yield on day 14 that maximises sensitivity and specificity to predict target yield **(³750ml by day 42**) is 518ml
Lussier 2019	Large cohort	Percentage of MOM during the neonatal unit stay was the best predictor of any HMF at discharge. For each 10% of MOM increase, there was a 93% increase in likelihood of HMF at discharge

Levene 2019	Small audit	Expressed milk yield **³750ml at mean 30 days of age** associated with higher **exclusive HMF at 1 month corrected** age, from 13% to 100% (p=0.03)
Hoban 2018a	Large cohort	Odds ratio of **any HMF at discharge** was 9.7 for achieving target yield (**³500ml/day by day 14**) 75% of mothers achieving target yield had any HMF at discharge, versus 36% of mothers who did not achieve target yield (p<0.001). Early milk yield was the strongest predictor for any HMF of all those studied
Hoban 2018b	Very small cohort	Achieving target yield (**³500ml/day by day 14**) associated with higher total milk expressed from day 1–5; 983ml compared to 167ml for mothers not achieving target yield (p=0.008)
Fewtrell 2016	Secondary analysis of small RCT	Expressing **>500ml at day 10** associated with higher mean yield/day throughout admission, from 178ml/day to 494ml/day (p<0.05) Weight of milk in first 10 days associated with mean yield per day throughout admission (p<0.001, r=0.84)
Morag 2016	Medium size cohort	Odds ratio of **exclusive formula feeding 6 weeks after discharge** was 3.3 for the infant having **<75% of enteral intake as MOM at day 14**
Omarsdottir 2015	Small cohort	Mothers of infants receiving **>80% of enteral intake as MOM from week 1–6** were already expressing enough milk to cover >90% of enteral intake by day 14
Wilson 2015	Medium size cohort	For babies born 28–31 weeks gestation, odds ratio of **exclusive HMF at 36 weeks' PMA** is 1.2 for each 10ml/kg of MOM at day 7
Murase 2014	Small cohort	Odds ratio for **formula feeding at discharge** of 7.1 for **day 4 milk yield <153ml/day** (p<0.01)
Hill 2005b	Small cohort	Expressed milk yield during week 1 associated with yield during week 6 (p<0.001, R^2=0.63)
Furman 2002	Medium size cohort	**Any HMF at 4 months corrected** age associated with higher yield at week 3, from 66ml/session to 126ml/session (p<0.001)

24. APPENDIX D: Summary of Patient Public Involvement (PPI) questionnaire

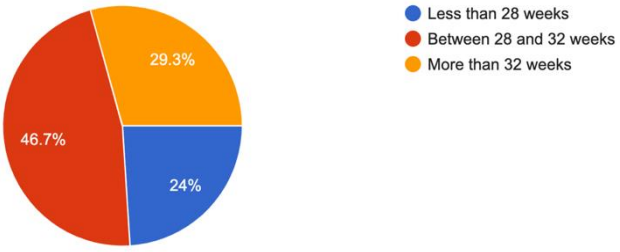
Aim: Explore mothers' feelings, problems and priorities around breastfeeding and expressing for preterm infants. Assess the risks and benefits of the planned study.

Method: An online survey was posted by Bliss and SSNAP on their social media channels in April/May 2019 asking for responses from mothers of preterm babies.

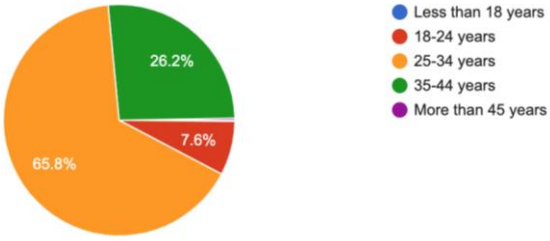
Results: 675 people responded to the survey.

Most mothers were White British, aged 25–34 years and had babies between 28 and 32 weeks gestation. There were a significant minority of respondents who were of black and ethnic minority background, <25 years old and had babies born <28 weeks, but face to face feedback from these groups will also be sought separately to ensure their voices are well represented. The length of breastmilk provision was very variable, showing that mothers with both 'good' and 'bad' experiences had responded.

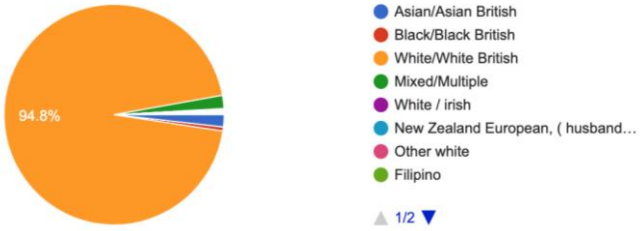
How preterm was your baby?



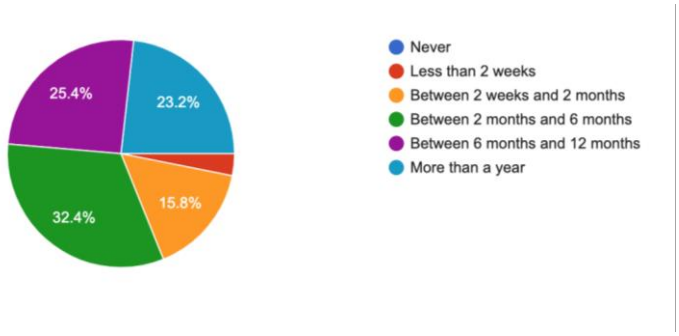
How old were you when your baby/babies were born?



What is your ethnic origin?



How long did your baby receive any of your milk?



1. **Did you have any problems expressing milk or breastfeeding?**

9 out of 10 mothers reported problems. The **most dominant theme was low expressed volumes/low supply**, expressed by one third of respondents. 1 in 10 respondents reported **stress and psychological difficulties.** There were no differences in problems reported based on gestation, age of mother or ethnicity.

One notable relationship was the **connection between stress and low supply**; the stress caused by time to milk coming in and colostrum volumes (many of which were actually in the normal range), the impact of stress on supply, the distress caused by low supply and the further impact on mental health caused by attempts to increase supply. Another theme was the impact of expressing on the rest of the mothers' lives, in terms of taking time away from caring for the baby and other children, looking after the mother's mental and physical health, and exacerbating or focusing the mind on the separation from the baby.

"My milk didn't come easily and having been told breastfeeding was best, but with no help on how to produce the milk I became so stressed and anxious about it that it started to affect my mental health"

"Endless hours of sitting alone in the dark with no child takes its toll on mental well-being. It was a very isolating experience, even though I knew I was doing the right thing"

2. **What questions would you like answered about how best to express for and breastfeed a preterm baby?**

Three quarters of respondents had questions. The most common question, expressed by 1 in 5 respondents, was **how to express more milk, particularly when physically separated from the baby**.

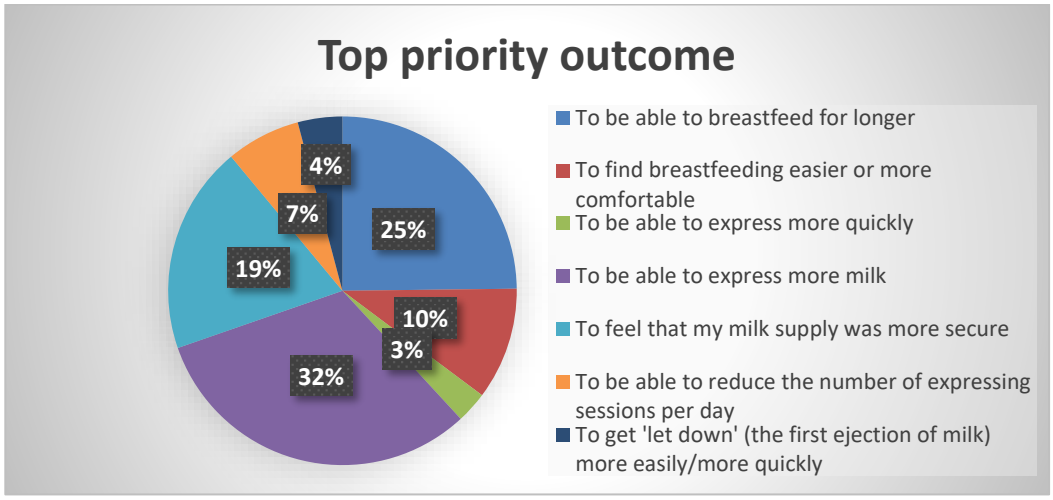
Around 1 in 10 respondents asked questions about **how often to express**, including whether night expressions are important for supply. Some mothers specifically wanted to know what **realistic frequency** of expressing to target, stating that the advice of 8–10 times a day was impossible or unrealistic

Around 1 in 20 respondents asked questions about **what expressed milk volume should be targeted over time, and whether there is a window of time to get supply established?** Around 1 in 20 asked **how to reduce stress, relax and increase connection with the baby while expressing.**

3. **What does success or improvement mean to you when thinking about breastfeeding and expressing for a preterm baby?**

84% responded with free text answers, which were very variable – there were no majority themes. The most common two markers of success for mothers (each reported by around 1 in 10) were feeling supported, and **being able to express more milk.** The next most common set of answers (around 1 in 20) were providing **exclusive maternal milk,** transitioning to direct breastfeeding, **a happy/relaxed mother, and breastfeeding for longer.** When volunteering a specific length of time that mothers were satisfied with or aiming to breastfeed there were three predominant groups – those describing 4–8 months, those describing 12–14 months and those describing 2–3 years.

When asked to rank a list of potential priorities, the dominant triad were **expressing more milk, more secure milk supply and breastfeeding for longer**. There were no significant differences in priorities in younger mothers (<24 years) or ethnic minorities. Although the top three remained the same across gestational age groups, more of those with the most preterm babies (<28 weeks) ranked expressing more milk as their top priority (37%) compared to those with the least preterm babies (>32 weeks, 24%, p = 0.007)



4. **Do you think there could be any negative effects of a research study looking at expressing mothers' milk and breastfeeding in preterm babies?**

On third of respondents had at least one concern, although 1 in 10 volunteered positive effects such as contributing to better support in the future, valuing your efforts in expressing, feeling like you were making a difference and distraction

The predominant concern was that mothers may have feelings of guilt, judgement, inadequacy, anxiety and depression in relation to expressing and breastfeeding and that being in a study where volumes are monitored and questions asked about breastfeeding outcomes could exacerbate this.

A concern from a smaller set of respondents was that the research study would be an extra burden, take time away from the baby or be intrusive, that an intervention could get people's

hopes up too much or that the results of the study could put further pressure on mothers to express/breastfeed or feel even more like a failure if they follow the advice and don't succeed.

Participants made suggestions to mitigate risks, which included:

- Communicating sensitively, respectfully and tactfully
- Ensuring that the description of the study does not put pressure on families but gives gentle encouragement, reassurance, support and empowerment
- To be explicit that many mothers have difficulty expressing and breastfeeding and this is "ok"
- Minimise any burden or interference
- Ensure that there is no incentive to express rather than breastfeed
- Don't attempt to recruit in the first few days of life

Although it wasn't mentioned in this section, one participant mentioned that expressing while grieving (when one twin has died) was "soul-destroying". Any potential exacerbation of this difficult time through inclusion in a study on expressing should be considered.

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