Statistical analysis plan for the Express-MOM study

Study title	Antenatal Breastmilk Expression from week 34 of gestation. Safety in pregnancy and benefits for the newborn infant
Acronym	The Express-MOM study
Registrations	ClinicalTrials.gov: NCT05516199 Ethics Committee of the Region of Southern Denmark: S-20210158 The research Ethics committee of University of Southern Denmark: 22/2533
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1. List of Abbreviations

aBME: Antenatal breastmilk expression

GA: Gestational age

MOM: Mother's own milk

DHM: Donor human milk

2. Statistical Principles

2.1 P values and confidence intervals

We will consider a P value of < 0.05 as statistically significant with a significant level at 95%.

2.2 Adherence to the intervention

We consider all participants who answer weekly questionnaires until 8 weeks post-partum as full completers. E.g. if participants try to follow the intervention as well as possible, but do not stimulate the breast the recommended fourteen times a week, we will still consider them as completers of the study, if they have stayed in the study during the intervention and until eight weeks after birth. However, we will differentiate between study completion and intervention completion. Intervention completion is defined as participants who follow the intervention as well as possible until birth but do not answer all eight questionnaires. These participants will be included in the analysis for gestational age at birth, but will only be included in the breastfeeding analysis as long as they have answered the weekly questions. If participants do not want to perform stimulation anymore, we consider this as a withdrawal of consent. If participants are not adherent to the randomized group, e.g. they start to stimulate antenatal even though they are in the control group, we consider them as excluded from the study.

2.3 Intention to treat (ITT) and per protocol (PP) analysis

We will apply a modified ITT principle, considering only the participants who started the antenatal stimulation and/or participated in the breastfeeding consultation and as the ITT population. Participants who drop out before the breastfeeding consultation for any reason will not be included in any of our analyses. We consider PP as all of the participants who completed questionnaires until 8 weeks post-partum, no matter how well they complied with the intervention. We will apply ITT and PP analysis to our primary and secondary outcomes.

3. Study design

3.1 Randomization

All participants will be randomized 1:1 into one of two arms: Group A aBME from week 34+0 or Group B no aBME. Randomization in this pilot trial will not be blinded to participants or investigators performing the data analysis due to the intervention type.

3.4 Withdrawal/dropout from the study

We will register the reason for withdrawal. We will divide withdrawal into the following categories:

1. Withdraw because of investigations in relation to the trial
2. Withdraw because of side effects in relation to the intervention
3. Withdraw because of time consumption in relation to the trial
4. Withdraw because of other reasons (will be described)
5. The participant is excluded from the trial because it is in the participant's best interest
6. The participant is excluded from the trial because the participant does not want to follow the instructions related to the randomization group
7. The participant is excluded from the trial due to loss of contact
8. The participant is excluded from the trial due to other reasons (will be described)

3.5 Time schedule

Recruitment was initiated on August 31, 2022, and will stop when 60 participants are enrolled.

Participants are enrolled and followed up continuously until 8 weeks PP

4. Analysis

4.1 Baseline characteristics

We will check the distribution for all reported data. The distribution will be checked by QQ plots and histograms. The normally distributed data will be reported as mean and standard deviations (SD) and the non-normally distributed data will be reported as median and interquartile ranges (IQR). Categorical data will be reported as numbers (n) and percentages (%).

Demographic data on mothers

- Age (years)
- Educational level
- Partner status

Clinical features on the mother

- Body-mass index (BMI) (kg/m^2)
- Height (cm)
- Pre-gestational weight (kg)
- Smoker status
- Medications (yes/no), if yes what type of medication and doses
- Gestational age at inclusion

Breastfeeding consultation

- Received other breastfeeding counseling (yes/no)
- Breastfed as a child (yes/partially/no)
- Partner participating in breastfeeding consultation (yes/no)

Only intervention group:

- CTG before stimulation (normal, deviant, or pathological)
- CTG under stimulation (normal, deviant, or pathological)
- CTG after stimulation (normal, deviant, or pathological)

Antenatal breastmilk expression (weekly questionnaires)

- Did you perform aBME this week (yes/no)
- If yes, how many times (amount of stimulations pr. week)
- Did you collect any milk (yes/no)
- If yes, how many milliliters (mL milk)

Clinical features on infants

- Gestational age at birth (weeks+days)
- Gender (boy/girl)
- Birthweight (g)
- Birth length (cm)
- Head circumference (cm)
- APGAR score at 1 min (0-10)

- APGAR score at 5 min (0-10)
- Enteral Nutrition PP if hospitalized
- Way of feeding PP if hospitalized
- IV-solutions PP if hospitalized

Breastfeeding rates up to 8 weeks PP (both groups, weekly questionnaires):

- Feeding the first 24 hours (Did your child get anything other than your milk, yes/no)
- Are you breastfeeding (yes/no)
- If yes (Partially or exclusively breastfeeding)

4.2 Outcome measurement

4.2.1 Primary outcome

Safety: the primary outcome is gestational age at birth in the two groups. Median and interquartile range will be used due to the non-normality of this variable and non-parametric test (Ranksum test), to detect differences between groups.

A posterior plot for the probability of non-inferiority will be produced by Bayesian binomial regression with symmetric weakly informative priors.

Adverse events will only be reported for the intervention group as numbers and percentages: Bleeding, regular contractions, uterus pain, Braxton Hicks contractions, or others that will cover anything else. Self-reported by the women in the intervention group.

4.2.2 Secondary outcomes

Breastfeeding rates

d) Proportion of exclusively breastfeeding infants vs. partially breastfeeding in each group at 24 hours, 1, 2, 4, 6, and 8 weeks post-partum, will be analyzed with mixed effects logistic regression (with restricted maximum likelihood) and visualized by a bar plot.

e) Proportion of any breastfeeding infants vs. formula in each group at 24 hours, 1, 2, 4, 6, and 8 weeks post-partum will be analyzed with mixed effects logistic regression (with restricted maximum likelihood) and visualized by a bar plot.

Antenatal breastmilk expression

a) Number of weekly expressions in the intervention group, measured through weekly push messages. Reported as median and IQR, and illustrated by a barplot figure over the number of weekly expression pr. women.

b) Amount of breastmilk expressed if any. How many women will be capable of expressing any milk before birth in the intervention group (counts and percentages), and how many mL (median and IQR). Volume is measured by each woman using a container with an mL scale and reported through weekly self-reported push messages. Milk volumes will be illustrated by a scatterplot over milliliter pr. week compared to the week of gestation.

4.5 Missing data

Missing data will not be imputated. We will only use the data available.

4.6 Statistical software

Statistical analysis will be carried out with Stata® statistical software