**TITLE: Effect of Antenatal Milk Expression on Breastfeeding Outcomes among Overweight and Obese Women**

**NCT Number: NCT04258709**

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**Recruitment:**

Potential participants may contact us to inquire about participation after learning about the study through various advertising strategies we may employ, including: 1) listing the study on the University research registry; 2) email advertisement via university email system; 3) study mailers (postal mail) sent to those identified via EMR as noted below; 3) study flyers posted in various recruitment locations; 4) advertising the study on hospital television monitors around the hospital; 5) advertising through local magazines; 6) utilizing study website through the University 7) advertising through social media posts, emails blurbs, newsletter blurbs or other communications by community businesses/organizations that serve birthing people, parents, infants, children, or families. Participant recruitment materials may also contain a QR code for participant ease. This QR code will be linked to a new record in our electronic data capture website where potential participants can leave their name and contact information (e.g., email, phone number to call or text) to receive more information about the study. We may also initiate contact with potential participants who appear to meet basic eligibility criteria according to select fields accessed within their electronic medical records. The study PI and study coordinators, who are considered part of the hospital-system covered entity by virtue of working under supervision of a co-I, may access the prenatal clinic patient lists in EPIC to assess potential patient eligibility (e.g., patients who are at least 18 years of age, will be coming into the clinic for their 34-36 week appointments, have no other children, and meet BMI criteria), with all other screening criteria assessed during screening after permission is given to approach/contact patient. Potentially eligible patients identified in this way (through EMR pre-screening) may then be approached in several ways, depending on clinic and hospital system regulations and preferences. 1. Clinical staff members at patients' prenatal provider's office may approach potentially eligible mothers at the prenatal visit to gauge interest in talking to study personnel about the study. If permission is granted by a mother, study staff may approach potential participant during/after prenatal visit to introduce study. Screening and enrollment may occur at the same time or later in-person or remotely. 2. Clinical staff or providers may give potentially eligible patients a study brochure and/or contact card. Note that clinical staff/providers may identify potentially eligible participants on their own or study staff may identify via EMR review and notify clinical staff to provide a patient a brochure/card. Interested patients may complete the contact card. Cards will be collected in line with practice guidelines (e.g,. left in patient room, returned to nurse, returned to front desk, dropped in a locked study box, etc.). Study staff will collect these cards and then contact interested patients via their preferred method to give them more information about the study and answer any questions. In lieu of contact cards, clinical research staff also may direct potential participants to reach out to study staff (either in-person if located at the hospital, or via phone or email). In clinical practices whose standard of care includes giving new pregnant patients folders at the time of their first visit, clinical staff may include a study brochure as this is a time where patients get many information opportunities including other research studies. Patients would then be able to contact study staff if interested and be re-contacted at 34 weeks of pregnancy, with their permission, to determine eligibility. 3. We may mail a study advertisement directly to their home address as noted in their EMR. The advertisement will include instructions on how to contact the study if potential participant is interested in learning more. Study staff may initiate contact/approach beginning at 28 weeks of gestation (other than the potential of distributing brochures in initial OB folders, which would occur as early as patients first prenatal appointment) to introduce the study (basic description, provision of study advertising or blank copy of consent form), with screening and enrollment to occur later between the 34th and 36th week of gestation. If the potential participant is agreeable, study staff may then track her future appointments in EMR and return during a prenatal visit at the clinic between 34-37 weeks gestation to again assess interest in study and complete screening procedures and enrollment if applicable. Alternately, with potential participant's permission, we will keep her contact information and

re-contact her using her preferred communication method (e.g., phone, text message) between 34-36 weeks gestation to arrange for in-person or remote screening and enrollment. This additional step of study introduction ahead of an enrollment visit may be necessary for some moms to have sufficient time to fully consider study participation and plan ahead for the time it would take to complete their enrollment procedures and baseline data collection. Note that we may mail study advertisements to potentially eligible patients identified through the EMR ahead of 28 weeks. If a patient does not provide consent to participate in the study, any protected health information obtained (e.g., maternal age, gestational age), as well as contact information, will be anonymized or destroyed.

Recruitment of prospective participants is detailed in the recruitment section. In all cases, once contact is made with a prospective participant, study personnel will provide a brief synopsis of the study, including study purpose, study procedures, potential risks and benefits, compensation and right to withdrawal from the study at any time without penalty. If interested in participating, study personnel will administer a brief screening form to the participant to ensure eligibility criteria are met. If ineligible, the participant will be informed of the reason for ineligibility and screening information will be retained without the participant's identifiable information (name, birthdate, etc.). If eligible, the research personnel will complete informed consent with the maternal participant. This informed consent may be completed in-person on paper, or remotely with a member of the research staff via Zoom (or similar platform) with an e-signature. Maternal participants will provide permission/consent for their infants to participate in the study, as infant-specific data, including background birth information and infant measurements, will be collected and the intervention has the potential to affect infant health. In all cases, maternal participants will sign and receive a copy (either hard copy or electronic copy) of the informed consent document. Any paper original copies will be kept and filed by research personnel. A separate addendum informed consent will be obtained to video-record AME sessions for interrater reliability. After informed consent, participants will complete baseline study measures/surveys (in-person, over phone or remote video/Zoom, or emailed survey; assessing maternal/fetal health, maternal demographics, maternal mood, reproductive history, body image concerns, experiences of discrimination, etc; see questionnaires for specifics) and be randomized to a group via computer-generated permuted block randomization stratified by pre-pregnancy BMI status (overweight: 25-29.9 or obese: ≥30). Subjects will have a 1:1 chance of being randomized to either study group, as indicated in the consent document. Study participation will be documented in the maternal participant's electronic medical record to alert providers to study participation. Study staff will meet remotely or in-person with participants in both groups weekly from 37 to 40 weeks of gestation for delivery of the assigned intervention. To maximize participant convenience and safety of all involved (given new standards/recommendations around social distancing) study "visits"/contact points will have the potential to be conducted completely remotely via a secure video call (e.g., HIPAA-compliant Zoom call with screen sharing when needed), audio phone call, or emailed survey. When/if feasible, preferred, and safe according to University research standards and current county-level guidance/restrictions, visits may also involve in-person meetings between the study participant and study staff (though the experimental condition lactation consultants will always deliver the intervention remotely). In-person visits may occur at the hospital/clinics before/after a prenatal visit or another mutually agreed upon convenient, private place. The details of study visits, according to assigned intervention are detailed below. Experimental Condition (AME): With the assistance of study staff, who will facilitate all study visits, participants randomized to AME will complete a test video call at enrollment, if possible, with the telelactation provider who are available for on-demand, one-on-one visits, 24/7) to increase familiarity with the service prior to AME instruction. Note that for subsequent study visits, if for any reason an IBCLC is not available, the study may use a substitute remotely-based IBCLC who is trained in study procedures. Participants will be taught the AME technique at the 37 week study visit after first viewing an

introductory video demonstrating the technique. At the visit, study staff will initiate a 3-way meeting/connection with an available, remote International Board-Certified Lactation Consultant (IBCLC) and the study participant if the visit is remote (e.g., Zoom video call). If the study visit is in-person, study staff will initiate remote 2-way connection between themselves and study participant with a remote IBCLC via a secure video platform (e.g., Zoom). After connecting, the remote IBCLC will provide video-based expert instruction and guided feedback on AME and milk collection as the participant engages in the technique. Note that IBCLCs are trained in hand-expression as a fundamental professional skill and will receive additional training and oversight by study personnel (see intervention fidelity below). For participants whose study visits are conducted completely remotely, these meetings will occur via a secure HIPAA-compliant platform like Zoom which allows study participant, study staff, and IBCLC to see/converse with each other simultaneously. This will require that participants use their own electronic device (e.g., smart phone, laptop) for the visit. These meetings will be scheduled a convenient time for the participant, IBCLC, and study staff. When a video-based remote connection is not possible or problematic (e.g., WiFi issues), study staff will initiate a 3-way audio only phone call instead with the IBCLC and participant, and hand expression education will occur over audio to the extent possible, with subsequent referral to supplementary streaming videos demonstrating hand expression. As per our pilot study protocol, AME will continue for a maximum of 10 minutes during study visits (participants will also be advised to do AME for no longer than 10 minutes during their independent practice between study sessions). For any in-person study visits, any milk output will be collected into study-provided containers and syringes and stored for future analyses as per the protocol outlined below (disposition of antenatal milk). During remote sessions, any AME milk that is collected may be labeled as milk that may be given to the study, and frozen by the participant. This milk may then be collected and aliquoted by study staff at a later date (e.g., no contact porch pick-up of milk at participant's home by study staff members). For all AME sessions, additional breastfeeding education will be provided by the IBCLC as requested by participant and documented by study staff. Study staff will facilitate the overall visit (e.g., introductions, provision of milk collection containers). IBCLCs will reinforce/provide feedback on AME technique at each weekly study visit until delivery, with a similar format to the initial visit. Around the time of the initial 37 week visit (this may be before or after the visit, dependent on mode of AME education delivery), study staff will provide participants oral and written instructions to continue AME 1-2 times/day at home until delivery. Instructions (reinforced in weekly visits) will also contain information on milk storage, transport to the hospital if participant is storing milk at home, and use; AME contraindications; and when and how to seek assistance for any problems (please see attached: brochures in "other" documents). Please note that for participants whose visits will occur remotely, members of study staff will drop off supplies for AME (e.g., collection containers, labels, syringes, refrigerator boxes for transport of milk to hospital) before the first scheduled visit, so that participants have supplies at the time of their first session. These will be done in accordance with any county standards in-place at the time (i.e., no-contact porch drop-off deliveries, PPE worn if any in-person contact). Compliance with home practice, including AME timing, milk volume obtained, and any problems, will be assessed from after the first study visit until delivery via daily web-based survey "diaries" (if participant declines web-based diaries or there are technical problems with web-based diaries, participants may be provided written diaries to be returned to study personnel at a time/place to be arranged, e.g., no contact porch pick up). Web-based survey diaries will be administered with an automated SMS notification sent daily to the participant with up to 3 reminders through the day to complete. The responses to web-based diaries will be automatically recorded in a secure Access study database; if participant completes written diaries, their answers will be manually entered in Access database by study staff. With regard to the SMS alert system for the web-based diaries, a two-way text messaging system has been built at the University and associated hospital for this purpose (as has the survey/diary system itself); the system utilizes a server

within the hospital system to manage all text message dialogue. The server stores all pre-specified libraries of assessment and contingent responses (e.g., automated responses specific to inputted information). The server will send queries or messages at specific times and schedules based on pre-specified study requirements. The server is connected to a modem which conducts all text message traffic. All incoming and outgoing text message dialogue is time stamped and recorded, accessible to investigators through a MS-Access system front-end. The server also has the capability to send email notifications of participant replies to investigators and allows the exchange of non-automated texts between participants and investigators. Participant phone numbers are entered into the system in two ways: 1. Participants text a specific code word into the modem phone number; 2. An investigator manually enters a participant's phone number into a web-based front end. Intervention Fidelity for Telelactation Visits: The study team will work closely with the telelactation clinical leadership to train IBCLCs in the standardized delivery of AME education (if other IBCLCs are used, they will also be trained in standardized delivery of the intervention). To verify IBCLC adherence to the study's AME teaching protocol, study staff will complete a checklist for each AME study visit (as well as checklists for control condition). For the first 20 participants receiving the AME intervention, interrater reliability assessment by the PI using the same checklist will occur for every fifth participant (all visits) who either 1) provides separate, written informed consent for video-recording visits for later review by the PI (in-person visits only), or 2) is agreeable to having the PI join the live remote session. Thereafter, depending on a satisfactory level of adherence to protocol, video reviews or live PI reviews may be decreased to every tenth participant for the remainder of the study. If participant visits are in-person, we will make an effort to avoid capturing identifiable features of a participant while filming (e.g., face) by aiming the camera at the study staff, although it remains possible that a participant may be visible or partially visible and their voice audible off-screen. If participant visits are remote, the PI will join the remote call, so that no recording takes place, and the study session checklist will be reviewed/marked in real-time. Disposition of antenatal milk: Similar to our pilot study, any milk samples collected during in-person study visits in pregnancy will be aliquoted, frozen, and stored at the University Biobank for later analyses (TBD) with participant permission. Any milk collected during remote study visits may be labeled as milk for research study and frozen in participant's home freezers. At participant and study staff convenience, this milk can later be given to study staff where it will be thawed, aliquoted, re-frozen, and stored at the University biobank for later analyses (TBD). If study staff arrange pick up of milk within 3-5 days of expressing, milk may simply be refrigerated by participants until the pick-up day, rather than frozen. Antenatal milk collected and stored by mothers at home outside of study sessions will be for use at their discretion (e.g., infant supplementation after delivery), and they will be provided with instructions on milk home storage, transport to the hospital, and use/provision to the infant. We will collaborate with hospital unit leaders at each birthing hospital to notify and educate unit staff who will potentially provide care for maternal and neonatal participants (e.g., nurses and providers in prenatal clinics, Labor & Delivery, postpartum, NICU) regarding our study and study procedures. A note will be placed in EMR alerting all providers to participants' study participation and potential existence of antenatal milk being brought in with participants at time of delivery and/or during postpartum hospital stay. Participants will be instructed to alert hospital staff during their birth hospitalization to their study participation and whether they have stored milk. Guidelines for safe storage and handling of antenatal milk will be communicated to participants, hospital staff, and posted on the refrigerated boxes provided to participants for milk transport to hospital.

Attention Control Condition (video-based infant care education): To stem threats to internal validity resulting from unequal attention between groups or lack of "treatment" incentivizing continued study participation, study staff will meet with control group participants weekly from 37 to 40 weeks of their

pregnancy, either remotely or in-person, similar to those in the AME group. In-person visits may occur at the hospital/clinics or another private mutually-agreed upon location; remote visits will be via a secure remote platform, such as the University's HIPAA-compliant Zoom. Visits will be approximately 10 minutes, similar to the intervention group. In this time, participants will view a standard set of web-based videos addressing evidence-based infant care, unrelated to feeding/breastfeeding. Videos will focus on a different theme each week (e.g., safe sleep, car seat safety, managing crying/colic), downloaded from free and reputable patient education sites. If visit is remote, study staff will attempt to "screen share" videos so that we may verify that participant actually views/receives the intended education (rather than directing participant to view videos on their own). Data Collection and Procedures Unrelated to Intervention Delivery: We will collect EMR data regarding maternal pre-pregnancy body mass index (BMI) and gestational weight gain, maternal and infant health conditions, labor and delivery information, and hospital infant feeding practices from the antenatal and postpartum medical records. Prenatal, intrapartum, and postpartum EMR data is housed within the hospital data systems, which the study team will access via their research and clinical affiliation with the hospital after IRB approval. In the postpartum period, study measures (surveys) assessing breastfeeding practices, perceptions, problems, satisfaction, plans, and education/support, as well as maternal mood, birth experience, childcare plans and use, and maternal employment (see questionnaires), will be completed by participants in both groups at weeks 2, 6, and 12 weeks, and 6 and 12 months postpartum. These measures may be completed remotely via emailed survey, in recognition of the time constraints faced by new mothers in scheduling study visits and calls. When difficulties are encountered in electronic survey completion, we will call participants or, when feasible, meet them during regularly scheduled postpartum clinic visits to complete measures (or at another mutually agreed upon time/location). If a participant elects to complete postpartum measurements and collection of a milk sample, the survey may be completed in-person at that time, as well. A subset of participants will also have included into their Baseline and postpartum surveys questions on sexual dysfunction. Please note that this survey is necessarily concurrent with a specific timepoint, and so participants who have already completed their participation in the study will not be asked to retrospectively complete this survey. In fulfillment of Aim 3, we will conduct individual telephone interviews with ≈25% of participants from both groups at 6 weeks postpartum, or earlier if they indicate that they discontinued breastfeeding at the 2 week postpartum survey or otherwise notify us at any point that they have decided to not start breastfeeding, do not initiate breastfeeding, or stop breastfeeding. We anticipate conducting interviews with approximately 70 participants, 35 from each group, selected purposively based on variability in AME uptake (e.g., some participants who did not complete any AME, some who did it once daily, twice daily, etc.), demographics, and current breastfeeding status to achieve a representative group). Interviews will continue until thematic saturation is reached (estimated at ≈25% of sample from our prior research). Interviews will address aspects of both AME and the control condition to maintain participant blinding to study purpose. Specifically, we will query participants on motivating factors for study participation; perceived benefits/utility of AME and antenatal video-based infant education; experiences with and evaluation of other breastfeeding and infant education support resources; experienced or anticipated burdens/challenges/drawbacks to AME and the control condition; and for AME participants, factors/contexts impacting AME uptake and use of antenatal-expressed milk, as well as suggested modifications to the AME intervention or study protocol. Interviews will be conducted by a qualitative research consultant or study personnel. Interviews will follow a semi-structured interview guide, adapted from our current AME pilot RCT, and may be modified as the study progresses based on themes raised in prior interviews or issues encountered by study personnel. Interviews are expected to last 30-45 minutes and will be audio-recorded and transcribed verbatim by QDAP, another secure (e.g., HIPAA compliant) transcription service, or study personnel. Both audio files and transcripts will be retained electronically without identifying information in accordance with IRB protocols.

## STATISTICAL DESIGN AND POWER

### Sample Size and Power

The total proposed enrollment of 280 women was determined considering a feasible rate of recruitment, expected effect size of the AME intervention on primary outcomes, testing of multiple hypotheses, and anticipated rate of attrition and protocol non-adherence at 2 weeks postpartum (primary outcome assessment). Our recent intervention research with normal weight and overweight/obese women demonstrates medium sized effects (standardized mean difference, d) for the AME intervention at 1-2 weeks postpartum compared to an education control in terms of higher levels of breastfeeding self-efficacy (AME: M=52.59, SD=9.92, Education: M=45.71, SD=15.71, d=0.53) and greater percentage of breast milk feeds (AME: M=89.50, SD=20.372; Education M=78.64, SD=27.303, d=0.45). Regarding exclusive breastfeeding at 1-2 weeks, rates of 0.27 have been reported among overweight/obese women.32 With a sample size of at least 210 (105 per group), we can detect with .80 power between-group differences in the proportions in breastfeeding exclusivity at 2 weeks postpartum as small as .172 (medium effect size of OR=2.55 using likelihood ratio chi-square statistics) at an adjusted test-wise significance level of .0167. While there is a fair amount of heterogeneity in the published effect sizes for breastfeeding support interventions among overweight and obese women and for AME specifically, our estimate lies in the range of reported effect sizes in the field.12,32 While the examination of long-term efficacy of AME is more exploratory (Aim 2), we will be able to detect small to medium interaction effects between groups over time as small as f = .323 when using repeated measures F-tests with at least four postpartum outcome time points. Our pilot work and previous studies utilizing similar populations and recruitment sites indicate that we can conservatively anticipate combined study attrition and intervention dropout at ≈25%. To ensure 210 participants with complete follow-up to 2 weeks and no protocol deviations, we will enroll 280 women.

### Statistical Methods

Preliminary Analyses: For Aims 1 and 2, detailed exploratory data analyses will first be performed, involving data description and data screening for anomalies (e.g., outliers, nonnormality, etc.). The results from this initial investigation will be used to: 1) describe

univariate and bivariate data distributions; 2) identify imbalances between treatment groups and associations between the dependent variables and suspected covariates/confounders; 3) evaluate the amount and patterns of missing data; and 4) check for violations of statistical assumptions for the planned analyses. If statistical assumptions are seriously violated, data transformations or more statistically robust methods will be considered. Covariates/confounders will be included in models secondarily, and their impact on the effect of the primary independent variable on the study outcomes will be evaluated. The randomness of missing data will be investigated using available information on subject characteristics to help discern patterns in the missing data, identify the possible covert missing data mechanisms, and inform the choice of the strategies to handle missing data. We will conduct logistic regression models to compare the characteristics of participants who remained in the study versus those who dropped out to determine if data are missing at random. If data are ignorably missing (i.e., not related to the outcome itself), likelihood estimation procedures, such as those in SAS PROC MIXED, GLIMMIX and NLMIXED as well as Mplus, will produce unbiased estimates while allowing us to retain cases with missing values on the outcome variables. If needed, multiple imputation would be used to impute missing values on the predictor variables. If data are not ignorably missing (i.e., non-randomly missing), we may use pattern mixture modeling to account for informative attrition.

Analysis Strategies for Aims 1 and 2: To examine the efficacy of the remotely-delivered AME intervention relative to the attention control on breastfeeding outcomes during hospitalization to the first two weeks postpartum (short-term; Aim 1) and up to the first 12 months (long-term; Aim 2) among overweight and obese (BMI ≥ 25) women, an intent-to-treat (ITT) approach will be used, where all participants will be included in analyses as randomized, regardless of protocol adherence/deviations, treatment received, or withdrawal. Adherence to the assigned intervention, however, will be monitored, and the sensitivity of the results under the ITT model will be explored to identify the effects of the amount of intervention received (e.g., number of AME study visits) and deviations in protocol on outcomes.

The primary outcome variables of interest for the short-term efficacy of the AME intervention at 2 weeks postpartum (Aim 1) are 1) proportion of mothers providing 100% breast milk, 2) percentage of feedings that were breast milk, and 3) breastfeeding self-efficacy. Secondary breastfeeding outcomes for short-term efficacy include proportion of mothers with any breastfeeding, perceived milk supply, onset and days to lactogenesis II, and days to any formula and/or breastfeeding cessation. Secondary long-term efficacy outcomes (Aim 2), which will be assessed at all follow-up points until 12 months postpartum (or breastfeeding cessation/loss to follow-up), are duration of breastfeeding in days and any breastfeeding at postpartum follow-ups; the continuous and categorical type versions of breastfeeding exclusivity; and the proximal intervening outcomes (i.e., mediating variables) including the repeatedly measured breastfeeding self-efficacy and perceived milk supply.

Generalized linear mixed-effects modeling with linear contrasts will be used to examine the effect of treatment assignment (AME vs. attention control) for each repeatedly assessed outcome, with treatment group assignment as the between-subjects factor, time as the within-subjects factor, and an interaction between time and treatment group. Given the level of measurement and observed data distribution of the particular dependent variable, an appropriate error structure and link function to the linear predictors will be assumed. For example, for breastfeeding exclusivity and any breast feeding, each assessed as a binary variable, a binomial error structure will be assumed and a logit link to the predictors will be applied, while for the continuous type versions of breastfeeding exclusivity and the interval-scaled breastfeeding self-efficacy, a normal error

distribution will be considered with an identity link to the linear predictors. Random effects for participants will also be included. Fixed and/or time-dependent covariates (e.g., pre-pregnancy BMI category [25.0-29.9; 3034.9; 35-39.9; 40+]) may be included secondarily to adjust for group imbalances or variables related to the dependent variables based on the literature or data screening results. Standard fit criteria (e.g., AIC and BIC) also will be used to identify the best-fitting repeated measures covariance structure. F-tests will test the main and interaction effects included in the model. Individual regression parameters will be estimated with confidence intervals. For each model, residual analyses will be conducted to identify sources of model misspecification, outliers, and influential observations. Sensitivity analyses will be performed to discern the impact of influential cases on modeling results. Linear contrasts will be specified in the repeated measures models to test whether the AME intervention demonstrates greater improvements in breastfeeding outcomes compared to the attention control at each time point, in particular, when conducting hypothesis testing on the primary short-term outcomes. Point and interval estimates will be obtained for each of the comparisons examined.

For event history type outcomes (e.g., breastfeeding duration, days to the onset of lactogenesis II, days to any formula) Cox proportional hazards regression methods will be applied to allow for possible censoring of the event of interest and inclusion of the fixed and time-dependent predictors. For summary outcomes (e.g., proportion of breast milk in-hospital), generalized linear modeling will be applied.

To explore possible moderators of the treatment efficacy of the AME intervention relative to the attention control, the generalized linear models for summary breastfeeding outcomes and the generalized linear mixed models for repeatedly assessed breastfeeding outcomes will be expanded to include the potentially moderating variable and its interactions with the other model terms (treatment group, time, treatment group by time). To explore possible mediation by the identified proximal/intervening variables (e.g., breastfeeding self-efficacy), mediational models will be fitted using structural equation modeling to allow for repeatedly assessed and censored proximal and distal outcomes, considering partial mediation and full mediation with no direct effects. From these analyses, path coefficients with confidence intervals will be obtained.

Analysis Strategy for Aim 3: We will undertake a qualitative analysis for Aim 3 (perceptions of and experiences with AME), described within the Research Strategy. We will also descriptively analyze characteristics of participants who participate in these interviews for reporting purposes.