Rev Esp Nutr Hum Diet. 2026; 30(1).
doi: 10.14306/renhyd.30.1.2569 [ahead of print]
Freely available online - OPEN ACCESS

Revista Española de Nutrición Humana y Dietética

Spanish Journal of Human Nutrition and Dietetics

INVESTIGACIÓN –*post-print version*

This is the version accepted for publication. The article may undergo stylistic and formatting changes.

Study of determinants of the nutritional composition of donor human milk: study protocol

Estudio de los determinantes de la composición nutricional de la leche materna donada: protocolo de estudio

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Received: 13/08/2025; Accepted: 11/12/2025; Published: 11/02/2026

Assigned editor: Evelia Aponiar Rodríguez, Hospital Regional de Alta Especialidad del Bajío, Secretaría de Salud, México.

CITE: Rola M, Dias CC, Pissarra S, Cardoso M, Silva D. Study of determinants of the nutritional composition of donor human milk: study protocol. Rev Esp Nutr Hum Diet. 2026; 30(1), 2569 doi: 10.14306/renhyd.30.1.2569 [ahead of print].

ABSTRACT

Introduction: Human milk is widely recognized as the optimal source of nutrition for newborns. In certain circumstances, such as in prematurity or illness, mothers' own milk (MOM) may not be available, and in such cases, donor human milk (DHM) is considered the preferred alternative. The aim of this study protocol is to investigate the influence of pasteurization, dietary habits and body composition of donors on milk composition.

Methodology: This study will include donors from the Human Milk Bank of the North at Unidade Local de Saúde São João who consent to participate. Sociodemographic, lifestyle and clinical data will be collected through questionnaires administered in interviews. Dietary intake of the donors will be assessed using a semiquantitative Food Frequency Questionnaire, validated for the Portuguese population. Donor´s height, weight, and fat mass will also be measured according to standard procedures. To assess the impact of pasteurization on nutritional composition, DHM samples will be analyzed before and after pasteurization with the Miris Human Milk Analyzer®.

Discussion and expected results: This research project, the first of its kind in Portugal, aims to elucidate the influence of specific factors on the nutritional composition of human milk. It is expected to generate valuable data that will enhance our understanding of nutritional composition of human milk and contribute to improving the quality of care provided.

Funding: no funding to declare.

Keywords: milk banks, human milk, food intake, pasteurization, body composition

RESUMEN

Introducción: La leche materna es ampliamente reconocida como la fuente óptima de nutrición para los recién nacidos. En determinadas circunstancias, como en casos de prematuridad o enfermedad, es posible que no se disponga de leche materna, y en tales casos, la leche materna de donada se considera la alternativa preferida. El objetivo de este protocolo de estudio es investigar la influencia de los siguientes factores en la composición nutricional de la leche materna donada: proceso de pasteurización, hábitos alimentarios de las donantes y composición corporal de las donantes.

Metodología: Este estudio incluirá a donantes del Banco de Leche Materna del Norte de la Unidade Local de Saúde de São João que hayan dado su consentimiento para participar. Se recopilarán datos sociodemográficos, de estilo de vida y clínicos mediante cuestionarios administrados en entrevistas. La ingesta alimentaria de las donantes se evaluará mediante un cuestionario semicuantitativo de frecuencia alimentaria, validado para la población portuguesa. También se registrarán la altura, el peso y la masa grasa de las donantes según los procedimientos estándar. Para estudiar el impacto de la pasteurización en la composición nutricional de la leche materna donada, se analizarán las amuestras antes y después de la pasteurización utilizando el Miris Human Milk Analyzer®.

Discusión y resultados esperados: Este proyecto de investigación, el primero de este tipo en Portugal, tiene como objetivo dilucidar la influencia de factores específicos en la composición nutricional de la leche materna. Este estudio generará datos valiosos para mejorar nuestra comprensión de la composición nutricional de la leche materna, lo que contribuirá para la optimización de la calidad de la atención prestada.

Financiación: No hay financiación que declarar.

Palabras clave: bancos de leche humana, leche humana, ingestión de alimentos, pasteurización, composición corporal

KEY MESSAGES

- Human milk is the optimal source of nutrition for newborns, and in mother's own milk absence, donor human milk is the best option.
- The nutritional composition of donor human milk varies according to several factors, including donor characteristics and the procedures involved in its processing.
- A comprehensive understanding of the impact of these factors on the nutritional composition of donor human milk is essential to improving the quality of services provided by human milk banks.

INTRODUCTION

The Importance of Human Milk

Human milk (HM) is widely recognized as the optimal source of nutrition for newborns, providing a comprehensive array of nutrients and bioactive compounds essential for optimal growth and neurodevelopment[1]. The benefits of breastfeeding are numerous and far-reaching, affecting the health of both the mother and the child. Documented benefits include the reduction of mortality and morbidity, and the prevention of overweight and obesity[1].

The composition of HM varies according to several factors, which adapt to the infant's age and other characteristics, to ensure optimal nutritional needs are met[2].

Donor Human Milk

In certain circumstances, such as prematurity or illness, mother's own milk (MOM) may not be available, and in such cases, donor human milk (DHM) is considered the preferred alternative[1,3]. When MOM is not an option, DHM is widely regarded as the optimal substitute, particularly for infants with a very low birth weight (VLBW ≤1,500 g) and for preterm infants[4].

It is important to note that DHM is not intended to replace MOM, but rather to serve as a crucial supplement in cases where exclusive MOM feeding is not feasible[5]. After preterm delivery, the availability of MOM is not guaranteed, as several mothers do not produce sufficient milk to meet their infant's needs[6].

The first human milk banks were created at the beginning of the 20th century, and at present there are approximately 280 such institutions across more than 20 European countries. In Portugal, the first milk bank opened in 2009 at the Maternidade Dr. Alfredo da Costa in

Lisbon. In 2022, the Human Milk Bank of the North (HMBN) was inaugurated at the Unidade Local Saúde de São João (ULSSJ). Since April 2023, HMBN has been certified under ISO 9001:2015 and is subjected to regular inspections.

Determinants of DHM Nutritional Composition

Several studies have reported growth retardation in infants fed with DHM, likely due to suboptimal nutritional composition[7, 8]. Despite the existence of numerous studies on the HM composition, findings cannot be generalized to DHM, since several factors contribute to changes in its nutritional composition. Among these factors are the diversity of donor characteristics (e.g., gestational age, stage of lactation, diet), inconsistencies in collection methods and the impact of milk processing[9].

Maternal obesity has been associated with an increased likelihood of gestational complications and adverse neonatal outcomes. Several lines of scientific evidence suggest an association between maternal obesity and alterations in HM nutritional composition[10, 11]. A systematic review found that maternal obesity was associated with changes in the concentration of fat and lactose in HM, which may stimulate weight gain and adipose tissue deposition in the neonate, thereby increasing the risk of obesity and metabolic dysfunction[12, 13].

Numerous studies have demonstrated that a mother's diet can influence the macronutrient composition of HM, particularly fat and fatty acids concentrations[14, 15]. This underscores the need for further research, as the majority of existing studies have examined only the association between specific nutrients or food intakes and HM nutritional composition. A recent European study by Binder et al. demonstrated an association between maternal diet and the nutritional composition of their milk, highlighting the importance of understanding this link, particularly in relation to the potential impact on the growth and development of preterm infants and the prevention of obesity[16].

DHM processing also alters its nutritional composition. Holder pasteurization (HoP) method is considered to offer the best balance between microbiological safety and nutritional quality[17]. Some studies have reported that pasteurization does not cause significant changes in certain nutrients, particularly proteins and energy value, while others have reported a decrease in fat and protein average concentrations[18-21].

Objectives

The aim of the present study protocol is twofold: firstly, to examine the impact of the donor's dietary intake and body composition on the nutritional composition of DHM; and secondly, to investigate the effect of the pasteurization process on the macronutrient composition of DHM.

2. METHODS

This study will include donors from the HMBN at ULSSJ who consented to participate. Consequently, the methodology described below encompasses the procedures conducted daily at the HMBN (Figure 1).

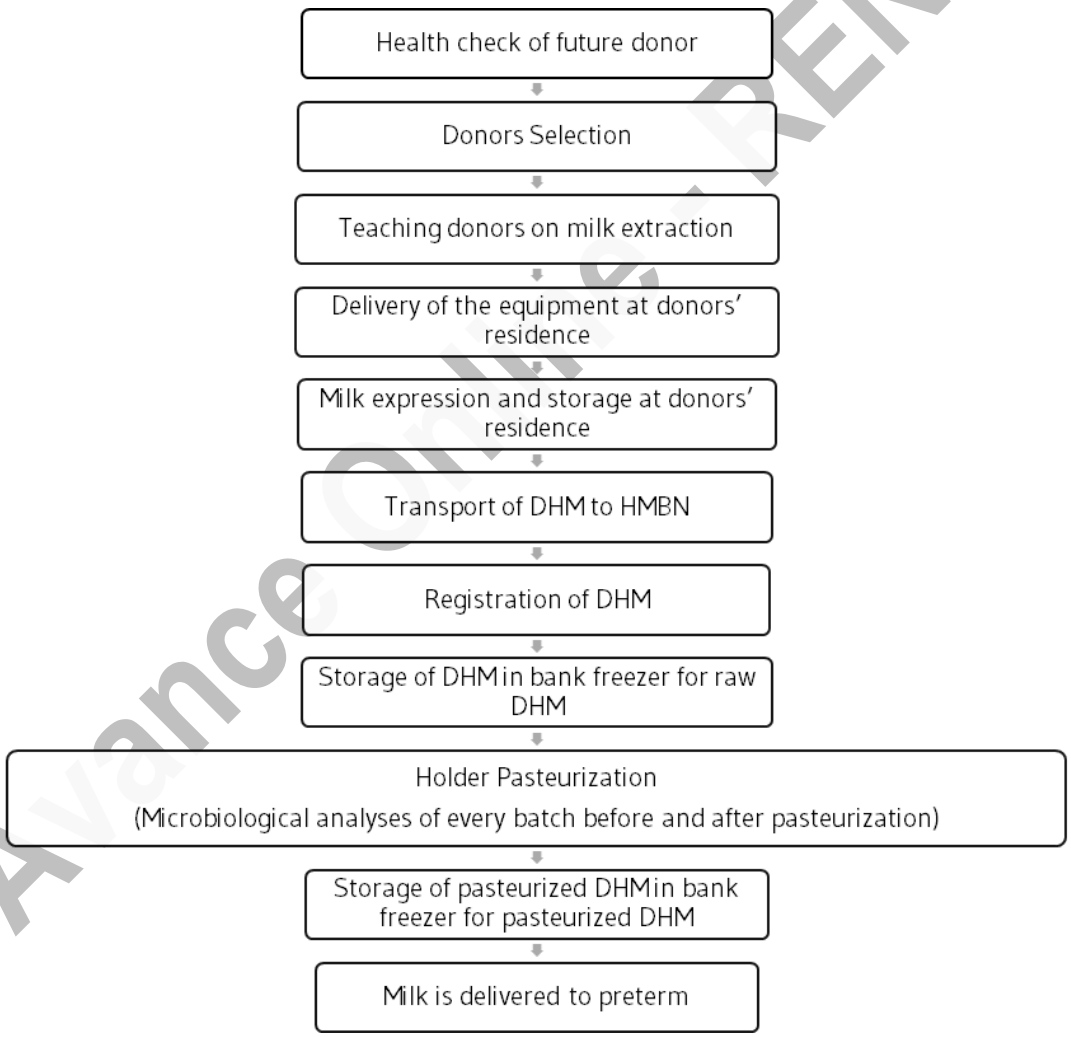

Figure 1. Flow chart of the human milk banking process.

Donors Selection

At HMBN, a rigorous human milk donor selection process is implemented to ensure milk quality and safety. A confidential questionnaire is administered to assess fulfillment of the necessary clinical criteria to become a donor. Women wishing to donate HM must meet predetermined criteria, namely: being a healthy lactating woman who, despite exclusively breastfeeding her child, has excess milk; being the mother of an infant under six months old; not smoking or consuming alcohol or caffeinated beverages; testing negative for HIV 1 or 2, Hepatitis B or C, Human T-cell Lymphotropic Virus I or II, and Syphilis; consenting to regular blood tests and accepting the conditions and rules of the HMBN.

If these criteria are fulfilled, a series of blood tests are performed on potential donors to screen for infections transmissible through milk, ensuring milk quality and infant safety. All the blood tests are performed at ULSSJ and the results are checked by the medical team.

DHM Collection

The HMBN team is trained with standardized guidelines and is responsible for training donors on procedures and care required for the at-home milk expression. The donor's home must meet certain hygiene conditions and must have easy access to a freezer. All donors are informed about the HMBN's telephone number and email address, allowing them to stay in close contact with the HBMN team.

A transport team delivers the necessary equipment to the donor's residence (electric breast pump, specific containers and labels for bottle identification).

Bottles used are pre-sterilized at HMBN and, according to safety protocols, must be used before the indicated expiration date. These containers are the sole approved receptacles for milk storage at donor´s home.

Milk Expression and Storage at Donor's Residence

After the extraction, milk must be immediately submitted to freezing (-18ºC to -20ºC) until delivery to HMBN. In the freezer, milk should be protected from potential contamination by enclosing it in a plastic box or bag.

Temperature must be closely monitored to avoid fluctuation that could compromise milk quality and safety. To ensure the accuracy of the recorded data, each donor is provided with

a temperature data logger, which tracks temperature variations during storage, ensuring the maintenance of the optimal temperature throughout the process.

Transport of DHM to HMBN

Once donors have utilized half of the bottles provided, they contact the transport team to schedule a pickup of the donated milk from their residence. DHM is collected from the donors' residences by an experienced transport team. During the transportation process, DHM is maintained at low temperature, under -18ºC to -20ºC, through thermic isolation with refrigerated containers, monitored by a temperature data logger, and the transport time must be less than 1 hour and 30 minutes.

Storage of DHM in HMBN

The HMBN team is responsible for the registration of DHM, assessing several parameters, including organoleptic characteristics (color and the presence of foreign bodies), bottle cleanliness, bottle tampering, signs of thawing, proper label completion, and temperature data recorded in the data logger. If any of the aforementioned parameters are found to be inaccurate, DHM is rejected. Accepted DHM is stored in the designated freezer for raw milk, awaiting pasteurization.

All the data entry is conducted within the MilkCENTER® system. MilkCENTER® is a Human Milk Bank Management Software that aims to manage milk donations in a human milk bank. It facilitates comprehensive monitoring and traceability throughout all stages of human milk processing, including the sending of containers to donors, the receipt of donations at the milk bank, pasteurization, storage, and the distribution of pasteurized human milk to services and hospitals. The software is designed to receive and store information regarding donors, including their suitability status. This ensures the traceability of the following stages: reception of DHM; pre-pasteurization; pasteurization; transfer and dispatch of batches of pasteurized milk; stock management and expiry dates. MilkCENTER® is integrated with the MilkTRAC® system, thereby enabling the seamless distribution of donated milk to services affiliated with patient care. This system facilitates the execution of closed-loop administration of DHM at the bedside, ensuring the precise and efficient delivery of nourishment to infants. The system functions by utilizing the unique identification bar codes printed on milk labels

and infant wristbands, which ensures rastreability of the milk distribution process and facilitate effective record-keeping.

Pasteurization

The DHM to be processed is thawed and stored at a temperature between 2 and 4°C for less than 24 hours. Subsequently, the milk is divided into batches, with each batch corresponding to milk from a single donor. Initially, the bottles of thawed milk are opened in a laminar-flow chamber, the contents of the different recipients are combined in a stainless-steel cup and the entire volume is filtered and homogenized. A microbiological control sample of 2mL is then taken from the mixture and transferred into the designated collection tube. The milk is then distributed into jars suitable for pasteurization, which are sealed with an aluminum seal at the chamber opening. The batch being processed is then identified.

The pasteurization method employed at HMBN is HoP. This method is endorsed by international guidelines, as it has been shown to preserve the beneficial and protective properties of HM[22]. HoP is characterized by a relatively low temperature (62.5ºC) and a prolonged heating time (30 minutes)[22].

After HoP, a compliance assessment is conducted in which a single bottle is selected to obtain a sample for subsequent microbiological and nutritional analysis. At HMBN, the microbiological screening criteria for DHM acceptance before and after pasteurization follows the Australian criteria[23].

The following methodology will be employed to address each study objective.

Nutritional Composition Analysis of DHM Before and After Pasteurization

The nutritional composition of DHM will be analyzed before and after pasteurization. To ensure accuracy, a 5 mL sample is obtained from each batch for analysis both prior to and following pasteurization.

Analysis will be performed using Miris Human Milk Analyzer® (Miris HMA®). The Miris HMA® is a device capable of determining the nutritional composition of HM with a minimal sample volume within approximately one minute, using a combination of established midinfrared transmission spectroscopy principles, exhibiting a high degree of reliability.

The analysis of DHM samples is preceded by cleaning and calibration of the Miris HMA®, according to the manufacturer's instructions.

Before analysis, DHM samples are warmed to 40°C and ultrasonically homogenized. Energy and macronutrient content are expressed in densities (kcal/dL for energy; g/dL for fat, raw and true protein, carbohydrates).

Interview with Donors

A questionnaire covering sociodemographic, lifestyle and clinical data was developed and will be administered during a clinical appointment at the Pediatrics/Obstetrics Service of ULSSJ. The questionnaire includes sociodemographic variables, such age, nationality, birthplace, educational level, occupation, and household composition.

Clinical data to be collected includes the number of pregnancies and births, and assessment of gestational diabetes, hypertension and pre-eclampsia in the most recent pregnancy. Data from the most recent birth, including gestational age, delivery mode, infant gender, and birth weight and length, will also be collected.

Lifestyle data encompasses information regarding physical activity and sleeping habits. Physical activity will be assessed through self-reported data on whether the donor practices any physical activity, what type of physical activity and the frequency (days and hours per week).

Regarding sleep habits, data will be self-reported on the number of hours of sleep the donor gets during the week and on weekends.

A comprehensive lactation and breastfeeding history will be recorded, including whether this is the donor's first breastfeeding experience, whether the infant is being exclusively breastfed, and breastfeeding frequency.

Donors' Dietary Intake Assessment

Dietary intake will be assessed during the same interview using a semiquantitative Food Frequency Questionnaire (FFQ), validated for the Portuguese adult population[24, 25].

The FFQ is a retrospective tool for assessing food consumption, including a pre-defined list of 86 food items. Consumption frequency is recorded in nine pre-specified categories ranging from ''never or less than once per month'' to ''six or more times per day''.

Consumption of each food item (in grams) is calculated by multiplying the frequency by the portion size reported. When appropriate, portion sizes may be adjusted for seasonal variation. Food intake will be converted into nutrient intake using a compilation of food nutritional composition databases: Food Processor Plus® (with nutritional information from the US Department of Agriculture's food composition tables, adapted to typically Portuguese foods), information from the Portuguese Food Composition Table, and other European sources and specific bromatological dosages of certain nutrients.

Body Composition of Donors

During the interview, anthropometric data will be collected. These include self-reported weight at the beginning and at the end of pregnancy, and the calculation of corresponding body mass index (BMI). Gestational weight gain will be calculated by subtracting the end-of-pregnancy weight from the beginning-of-pregnancy weight. BMI classification will follow WHO cut-off points, and adequacy of gestational weight gain will be classified according to the Institute of Medicine recommendations, adjusted for pregnancy duration[26, 27].

Anthropometric measures will also be obtained following standard procedures[28]. Height will be measured in centimeters (with 0,1 cm of resolution) using a SECA 799 ultrasonic measuring station. The measurement is taken with the donor standing, with feet and knees together and head in the Frankfurt plane[28].

Body weight (Kg), and fat mass (%) will be measured using the InBody270S bioimpedance scale. The measurement is taken with the donor barefoot and wearing light clothing, without jewelry or heavy accessories. The donor stands on the scale with bare feet and holds the electrodes; she must remain still during the process. BMI will be calculated and categorized according to WHO classification[26]. Waist and hip circumferences will also be measured and analyzed according to WHO criteria[29]. The measurement of waist and hip circumference is taken with the donor in a relaxed standing position with the arms folded across the thorax. The waist circumference will be measured at the level of the narrowest point between the lower costal (10th rib) border and the iliac crest[28]. The hip circumference will be measured at the level of the greatest posterior protuberance of the buttocks[28].

Statistical Analysis

The study sample size was calculated based on the results obtained by Lopes da Silva *et al.* in the pilot study[30]. Therefore, the sample was estimated considering an effect of 0.28 on the post-pasteurization fat value and a significance level of 0.05 and 80% power, thus, we estimated a required sample of 100 donors.

A database will be created and statistical analyses will be performed using IBM® SPSS® Statistics, version 30.0 (Statistical Package for the Social Sciences).

Initially, the distribution of continuous variables will be assessed to determine the most appropriate statistical tests using the histogram or the Kolmogorov-Smirnov test. Descriptive statistics will be used to characterize the variables: categorical variables will be summarized using absolute and relative frequencies, while continuous variables will be reported as mean and standard deviation or as median and interquartile range, depending on the data distribution.

Associations between variables will be evaluated using parametric tests (T-test for independent samples or ANOVA test) or non-parametric tests (Mann-Whitney or Kruskal-Wallis), as appropriate. Hypotheses regarding categorical variables will be tested using a Chi-square test or a Fisher's exact test, as appropriate.

To have a more thorough understanding of the factors associated with the outcomes defined, multivariate linear or logistic regression will be performed. Potential confounding factors will be considered, such as lactation time, gestational age, donor age, parity, and type of delivery. The coefficient regression (beta) or odds ratio (In (beta)) associated with 95% confidence intervals (95% CI) will be presented.

All models will be built using a stepwise selection approach. Model goodness-of-fit will be assessed using the Hosmer–Lemeshow test, and discriminative performance will be evaluated using receiver operating characteristic (ROC) curve analysis for logistic regression and coefficient of determination (R^2) for linear regression.

In all statistical tests, a level of statistical significance of α=0.05 (5%) will be considered.

Ethical Issues

The present study was approved by the institutional ethics committee (no.88/2024). Recruitment requires written informed consent obtained from the donors. The study is registered at www.clinicaltrials.gov ID: NCT06904924.

3. DISCUSSION

This prospective cohort study will evaluate the effect of three factors on DHM nutritional composition: pasteurization, donors' dietary habits and donors' body composition. The study will include healthy lactating women who were recruited at HMBN. Data will be collected not only on maternal diet and body composition, but also on weight gain during pregnancy, physical activity and sleeping habits, which may also influence DHM nutritional composition.

For the study of donor's dietary habits, the FFQ, which is a valid and reliable tool for assessing nutrient consumption, will be applied by an interviewer. The FFQ version applied by an interviewer has an extra section for recording the portion consumed (equal to, greater than or less than the standard average portion described). This survey has been validated for the adult population by comparing the information reported in it with 7-day food diaries collected at four different times of the year and with adipose tissue samples from the buttock region [25]. Conversion of food consumption to nutrients intake will use a compilation of food nutritional composition databases, including information from the Portuguese Food Composition, improving nutrient intake estimates given country-specific differences in food composition.

Anthropometric measures will follow the International Standards for Anthropometric Assessment. Body composition analysis will be assessed using the InBody270S bioimpedance scale, a non-invasive and rapid method considered convenient for donors. To minimize the limitations of this method, the same equipment will be used throughout the study, and measurements will be taken at the same time of the day.

HM is a comprehensive source of macro- and micronutrients and bioactive factors essential for infant growth and development. Nutrient composition and volume of expressed HM vary between and within mothers. To reduce intra-individual variability, milk from each donor is mixed and homogenized.

Nutrient composition of HM changes with the nutritional status of mothers, including food consumption and body composition [12, 16, 31-33]. However, the relationship between these factors is not consistently reported, and, hence, further investigation is required.

Nutrient composition analysis of DHM will be performed using the Miris HMA®, which is a valid method [34, 35].

There are several limitations of the study that are described below. First, the feasibility of the study depends on recruitment of a sufficient number of donors for the HMBN who are willing to participate in this study. Second, nutritional composition assessment of DHM will be carried out on a single donation. Third, the use of the FFQ as a substitute for food diaries, which are the reference method, may also represent a limitation. However, given the characteristics of the study population, it is probable to observe a low response rate if food diaries are employed. Finally, the participants are likely healthier than the general population because they were recruited as DHM, so caution is required when extrapolating results to the general population. It should be noted that according to the characteristics of the study, selection bias is to be expected, more specifically volunteer bias, which has been described in several studies as being more likely to occur in this type of sampling. All data collected will be used to develop a model. There are currently no plans for external validation, as this is a project for the future.

4. CONCLUSIONS

Previous research has demonstrated the impact of several factors on HM nutritional composition. This research project, the first of its kind in Portugal, aims to elucidate the influence of specific factors on DHM nutritional composition, including donors' body composition and eating habits, as well as the impact of pasteurization. The study will generate useful data to improve our understanding of DHM nutritional composition, leading to improvements in the quality of care provided. It is imperative to understand the influence of these factors on the nutritional profile of DHM, in order to optimize its benefits for vulnerable infants. This is of pertinence in the context of the management of preterm infants, and it can be employed to enhance growth rates and facilitate optimal development. In addition, the knowledge acquired through this study can be applied to breastfeeding mothers, with the goal of promoting their children's health.

AUTHORS' CONTRIBUTIONS

All authors contributed to the writing and editing of the study protocol.

FUNDING

The authors declare that no funding was received for the conduct of this study. All the material and human resources will be provided by the financial resources of the Unidade Local de Saúde de São João.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding writing this manuscript.

STUDY REGISTRATION

The study is registered at www.clinicaltrials.gov ID: NCT06904924.

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