SCIENTIFIC PROGRAMME

emba
EUROPEAN MILK BANK ASSOCIATION

3rd INTERNATIONAL DONOR MILK RESEARCH CONGRESS

NEW PERSPECTIVES AFTER THE COVID-19 ERA

MILAN
May 13th, 2022

event organized by | BioMedia
The sharing of knowledge

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SCIENTIFIC PROGRAMME

8:30 am **Welcome**
Enrico Bertino

8:40-9:30 **EMBA WORKING GROUPS**
Coordınator: Enrico Bertino

GUIDELINES
Gillian Weaver

PROCESSING OF DONOR HUMAN MILK
Guido Moro

HUMAN MILK FORTIFICATION
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FUNDRAISING
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THE IMPACT OF MATERNAL DIET ON DONOR HUMAN MILK
Aleksandra Wesolowska

INDICATIONS FOR THE USE OF DONOR HUMAN MILK
Jean-Charles Picaud

MICROBIOLOGY OF DONOR HUMAN MILK
Corinna Gebauer

CULTURAL AND ETHICAL ASPECTS IN MILK DONATION ACCEPTANCE
Sertac Arslanoglu

9:30-10:30 **Invited Speakers' Conference**
Moderators: Sertac Arslanoglu, Jean-Charles Picaud

Not just about the baby; DHM and maternal mental health
Amy Brown (Swansea, UK)

The HAMLET story - from the lab to clinical trials
Catharina Svanborg (Lund, Sweden)

10:30-11:00 Coffee Break

11:00-11:20 **Survival of a Human Milk Bank in Kiev during Russian aggression against Ukraine**

Valery Barysznikowa, Human Milk Bank in Perinatal Center in Kiev (Kiev, Ukraine)

Introduction by
Aleksandra Wesolowska

11:20-13:00 **Oral Communications**
Moderators: Gillian Weaver, Clair-Yves Boquien

OC 01 Biovigilance in Milk Banking
V. Clifford (Melbourne, Australia)

OC 02 Establishment of first Human Milk Bank in Georgia
N. Solomonia (Tbilisi, Georgia)

OC 03 Effect of Lactobacillus bacteria obtained from breast milk on MCF -7 cells in vitro
K. Lubiech (Bydgoszcz, Poland)

OC 04 Metabolomics characterization of preterm human milk in the first month of lactation: From extremely to moderate prematurity
F. Cesare Marincola (Cagliari, Italy)

OC 05 Impact of pooling strategies on macronutrient variability of pasteurized donor human milk
C. Tabasso (Milan, Italy)

13:00-14:00 Lunch

14:00-15:00 **General Assembly**
(including new Board election)

15:00-16:40 **Oral Communications**
Moderators: Corinna Gebauer, Guido E. Moro

OC 06 Development of a cellular-based assay for measuring the anti-inflammatory potency of donor banked milk
L. Loubaki (Québec, Canada)

OC 07 "One of my biggest breastfeeding goals was to donate milk": Sociological and cultural considerations of human milk donation
T. Cassidy (Dublin, Ireland)

OC 08 Overview and preliminary findings from a global study to characterize nutrients in donor human milk
A. Wesolowska (Warsaw, Poland)

OC 09 Vegan/Vegetarian diet and human milk donation: An EMBA survey across European milk banks
S. Gandino (Oxford, UK)

OC 10 Mother's milk in Chronic Kidney Disease: A case-control study
M. Giribaldi (Grugliasco, Italy)

16:40-17:00 **General Discussion**

17:00 **Closing remarks from the new President**

GENERAL INFORMATION

CONGRESS VENUE
BIOMEDIA Torre U8 – Second floor
Via Libero Temolo 4
20126 Milano

CERTIFICATE OF ATTENDANCE
All attendees will receive the certificate of attendance

ORAL COMMUNICATIONS

OC 01
BIOVIGILANCE IN MILK BANKING
V. Clifford, L. Klein, I. Gosbell, R. Brown, V. Hoad, J. Pink
Australian Red Cross Lifeblood
Murdoch Childrens Research Institute
University of Melbourne

Biovigilance is a surveillance system for monitoring adverse events in donors and recipients associated with the use of biological products.
Although the regulatory status of donor milk varies internationally, donor human milk is considered a medical product of human origin by the World Health Organization, making it incumbent on milk banks to establish and maintain biovigilance systems. Although Holderpasteurised donor human milk is generally a low-risk product, biovigilance systems are of particular importance when evaluating alternative processing techniques for donor milk or other human-milk derived products such as fortifier.
Australian Red Cross Lifeblood Milk was founded in 2018 and now supplies pasteurised donor human milk to more than 30 hospitals across Australia. Since inception, Lifeblood has done ongoing prospective surveillance for milk donor and recipient adverse events. Over this time, we recorded three donor adverse events (3/976= 0.31%) related to phlebotomy; these included two vasovagal reactions and one phlebotomy site haematoma. Eight (8/976= 0.81%) additional donors had biological false reactive infectious diseases serology results, which are not donor adverse events, but have potential to disrupt the breastfeeding relationship between the donor and her own infant. No recipient adverse events were attributable to donor human milk. Whilst 10 suspected adverse events in recipients were reported to Lifeblood, all were eventually classified as unlikely to be related, or definitely not related, to donor human milk.
Strong biovigilance systems offer several short-term and long-term advantages. Patient safety is improved by early detection of disease transmission. Other significant advantages include detailed information to improve and refine donor selection criteria, increased knowledge and communication between providers and prescribers of donor milk and valuable information to inform development of processing guidelines and standards. We will discuss potential opportunities for collaboration in establishing formal biovigilance systems in milk banking globally.

OC 02

ESTABLISHMENT OF FIRST HUMAN MILK BANK IN GEORGIA

N. Solomonia[1], E. Kandelaki[2]
[1]*M.Iashvili Children's Central Hospital, Tbilisi, Georgia*
[2]*Tbilisi State Medical University*

Country of Georgia, with population of 3.7mil. birth date 47000 /y. Over the past decades, Perinatal care has much improved in Georgia and perinatal mortality rate significantly decreased. Iashvili Children's Central hospital, Tbilisi, Georgia, operates countries one of the biggest level III- IV, 60 bed Neonatal Intensive Care Unit. 600 patient admission rate per year. It's also the countries major center for surgical neonatal patients. Hospital has been pioneer in establishing Neonatal health care standards: Parenteral Nutrition, implementing PICC, promotion of breastfeeding, initiation of early minimal enteral feeding in preterm infants, human milk fortification in Preterm infants, therapeutic hypothermia... etc. Unfortunately, no donor milk available, for high risk infants .Since 2019, has been tremendous work done to Increasing awareness around the benefits of mother and donor milk, National guidelines for high risk infants enteral feeding has been developed. 2021, Human Milk pilot project has been designed, in order to establishing first human milk bank in Tbilisi, Georgia, at M. Iashvili Children's Central hospital, NICU. Project has won a grand prize, in Innovation Idea Contest and given full financial support by EVEX Medical Corporation, in December 2021. In the beginning, we are planning to establish, human milk bank to supply infants at the M. Iashvili Children's Central Hospital NICU. Target group for the donor milk supply: Newborn infants < 1500g and all ill neonates with digestive problems. Financing: at this time 100% of human milk bank will be financed by Evex Medical Corporation and M.Iashvili Children's Central Hospital.Pilot project will serve as a starting point for developing national human milk bank guidelines and establishing a human milk bank network throughout the country and Caucasus region, with the potential to save thousands of lives and ensure healthy growth and development.

OC 03

EFFECT OF LACTOBACILLUS BACTERIA OBTAINED FROM BREAST MILK ON MCF -7 CELLS IN VITRO

K. Lubiech, M. Twaruzek
Dep. of Physiology and Toxicology, Faculty of Biological Sciences, Kazimierz Wielki University, Bydgoszcz, Poland

Human milk is the optimal food for infants and toddlers. The composition of breast milk changes over time and adapts to the needs of the developing organism, satisfying nutritional needs at an early stage of growth and development. An important component of breast milk is probiotic microflora. Our previous research has shown the presence of Lactobacillus bacteria in breast milk only some breastfeeding women's - approximately 20% of the examined breast milk samples. Breastfeeding is beneficial not only for the baby but also for the mother. Our research is a part of wider research on Lactobacillus bacteria obtained directly from breast milk and focuses on the various properties of bacterial strains.
The aim of the study was to evaluate the effect of Lactobacillus bacteria, strains isolated from breast milk, on breast cancer cells of the MCF-7 line in vitro.
The research was carried out on strains previously isolated from breast milk samples. The technique of MTT test was conducted. We used bacterial supernatant of each strain to investigate the effect of Lactobacillus bacteria products on viability of MCF-7 line cells.

OC 04

METABOLOMICS CHARACTERIZATION OF PRETERM HUMAN MILK IN IHE FIRST MONTH OF LACTATION: FROM EXTREMELY TO MODERATE PREMATURITY

F. Cesare Marincola[1], C. Peila[2], M. Stocchero[4,5], S. Sottemano[2], A. Dessi'[3], E. Baraldi[4,5], V. Fanos[3], E. Bertino[2]

[1]Dep. of Chemical and Geological Sciences, University of Cagliari, ITALY
[2]Neonatal Unit, University of Turin, City of Health and Science of Turin, Turin, Italy
[3]Neonatal Intensive Care Unit, Neonatal Pathology and Neonatal Section, Azienda University Polyclinic, University of Cagliari, Cagliari
[4]Institute of Pediatric Research (IRP), Fondazione Città della Speranza, Padova, Italy
[5]Department of Women's and Children's Health, University of Padova, 35128 Padova, Italy

Human milk (HM) is the best way to feed a newborn in the first months of life as it contains a balanced amount of nutrients and bioactive components which guarantee optimal growth and development of the organs, immune system, and intestinal microbiota. During the past decade, a particular attention has been turned toward the HM metabolome that is the pool of low molecular weight (<1.5 kDa) metabolites associated with gene expression and protein activity. Although the HM metabolome composition is known to be related to maternal characteristics as genetics, diet, and lifestyle, as well as gestation age (terms vs. preterm delivery), type of delivery, and lactation time, its characterization remains incomplete, and its role in the infant nutrition is not totally understood.
In this study we contributed to the advance of knowledge of the composition of the preterm (PT) HM metabolome. By a metabolomics approach based on Nuclear Magnetic Resonance (NMR) spectroscopy, we analyzed the metabolic profiles of HM samples collected in the first month post-partum from mothers (n = 36) delivering extremely (n = 14), very (n = 11) and moderately (n = 11) premature infants. Compositional variability was analyzed in terms of the degree of prematurity and lactation stage. We observed that the more preponderant compositional variations were due to mother's phenotypes and lactation stage. A significant, although weak, impact of GA was also noted comparing samples taken from mothers with extremely and moderately preterm delivery: the levels of 1,3-linked fucosyl residues, 3'sialyllactoses, choline, myoinositol, and glucosyl moieties in the metabolome of moderately preterm milk were higher than in the extremely preterm group. This result suggests that the PT milk metabolome differs in its metabolite composition depending on the degree of prematurity. Further research should be done to confirm these findings and understand their biological importance.

OC 05

IMPACT OF POOLING STRATEGIES ON MACRONUTRIENT VARIABILITY OF PASTEURIZED DONOR HUMAN MILK

C. Tabasso[1], P. Piemontese[1], D. Mallardi[1], N. Liotto[1], C. Menis[1,2], M. Perrone[1], O. Amato[1], A. Orsi[1], N. Pesenti[1,3], P. Roggero[1,2], E. Bezze[1], L. Plevani[1], F. Mosca[1,2]

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[2]Department of Clinical Science and Community Health, University of Milan, Milan, Italy

[3]Department of Statistics and Quantitave Methods, Division of Biostatistics, Epidemiology and Public Health, University of Milan, Italy

Background: Donor Human Milk (DHM) is recommended feeding for preterm infants when mother's own milk is unavailable or insufficient. The well-known variability macronutrient content of DHM may have significant implications on preterm infants' feeding. In human milk banks two different strategies of pooling DHM (target and random), could be useful in reducing macronutrient variability, facilitating the achievement of nutritional requirements of preterm infants, reported by the ESPHGAN guidelines.

Aim: The impact of two different pooling strategies on Pasteurized Donor Human Milk (PDHM) variability was evaluated.

Methods: In our human milk bank 1169 single donor pools of PDHM collected by 409 donors, were analyzed using a human milk analyzer. Thus, a target pooling strategy (TP) combining 2-3-4 or 5 single donor pools of PDHM, was adopted. Secondly, on the bases of single donor pools' analyses, a test of 10.000 randomly simulated pools (RP) for each combination of donor considered, was performed.

Results: The comparison between two different pooling strategies revealed that the percentage of pools, which have a concentration of fats < 2,5g/dl, is higher for RP than those obtained by TP, especially considering 2 or 3 donors/pool. The same result was obtained for proteins considering the concentration of 0,7 - 0,8g/dl and for energy considering the concentration of 53-58 Kcal/dl. No pool had a concentration of proteins < 0,7 g/dl, independently from the type of strategy used.
As concerns 5-donors pools, RP and TP strategies could be considered comparable in terms of percentage of pools which macronutrient content was greater than or equal to reference values, except for protein.

Conclusion: When a TP strategy was not feasible it should be useful to perform a RP strategy combining at least 5 donors, in order to reduce the variability of PDHM and facilitate the achievement of nutritional requirements of preterm infants.

OC 06

DEVELOPMENT OF A CELLULAR-BASED ASSAY FOR MEASURING THE ANTI-INFLAMMATORY POTENCY OF DONOR BANKED MILK

C. Marie-Lee[1,2], P. Isabelle[1], L. Lionel[1,2], C. Steve J[2,3,4]
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[2]Department of Biochemistry, Microbiology and Bioinformatics, Laval University, Quebec City, QC, Canada
[3]Institut de Biologie Intégrative et des Systèmes, Laval University; Quebec City, QC, Canada
[4]Centre de Recherche de l'Institut Universitaire de Cardiologie et de Pneumologie de Québec, Quebec City, QC, Canada

Background: Meeting extremely preterm infant nutritional needs are one of the main goals of the human milk feeding strategy in the treatment of necrotizing enterocolitis (NEC). Bioactive components of human milk also play an important role in the prevention of NEC. Thus, the objective of our study was to develop an assay to assess anti-inflammatory potential of specific milk samples (DHMs) through the monitoring of NFκB activation, a key factor that regulates inflammation. Methodology: Small intestine epithelial cells (FHs74) were seeded in 24-well plate and left to adhere for at least 18 hours after which the medium was removed and cells were incubated with DHMs or controls for 2h. Then, cells were washed with saline prior to the addition of peripheral blood mononuclear cell supernatant used as NFκB activator. After the stimulation, cells were recovered and the activation of NFκB was measured by flow cytometry or by ELISA.
Results: Using this model, we were able to identify DHMs with strong, moderate and no anti-inflammatory ability. Variation among the triplicate in the same assay was less than 5% and variation between different assay performed at separate times was ≤10% supporting the reproducibility of this assay. Conclusion: Coupled to nutritional information, this potency assay could help breast milk banks or neonatal intensive care unit personnel improve their strategy during the selection and preparation of breast milk batches and favor a product with high clinical efficacy for the preterm infant.

OC 07

ONE OF MY BIGGEST BREASTFEEDING GOALS WAS TO DONATE MILK": SOCIOLOGICAL AND CULTURAL CONSIDERATIONS OF HUMAN MILK DONATION

Tanya M. Cassidy
School of Nursing, Psychotherapy, Community Health (SNPCH)
Dublin City University (DCU)

There is a lack of sociological and cultural discussion regarding donor human milk, although there have been far more discussions of breastfeeding and the underlying features of motherhood, mothering, or more recently parenting. By exploring previous sociological and cultural research on donor human milk services, we will discuss various methodological considerations and concerns, arguing for the value of detailed qualitative research, rather than the use of alternative traditional survey methods, a choice which resonates within sociological and anthropological disciplines more generally.

Our methodological discussion will turn to the value of triangulated qualitative data collection, which ideally can be analysed using pragmatic frames from the Chicago tradition of abductive analysis, less well known in health research, but recently recuperated to discuss conjunctive theorising and rapid policy making during the pandemic.

Our discussion with then turn to a reoccurring theme in qualitative research on donor human milk services, and look at the specific question: 'Does donor human milk act as a bridge to breastfeeding or a substitute for breastfeeding?' Ethnographic work from the world of donor human milk services suggests that the protection, support and promotion of breastfeeding is an often taken for granted aspect of donor human milk services, following the truism that these services are literally dependent on lactation to exist. Triangulated with historical data and interviews, particularly from Ireland and Scotland, we look at the changes associated with donor human milk services in the 21st century, and the increasing expansion of links between this service and breastfeeding policies and practices, concluding with a discussion regarding social and cultural considerations regarding donor human milk service which requires not merely biomedical research into the properties of donor milk, but informed social and cultural research enabling policy makers to better understand how and why human milk services are needed and should be supported.

OC 08

OVERVIEW AND PRELIMINARY FINDINGS FROM A GLOBAL STUDY TO CHARACTERIZE NUTRIENTS IN DONOR HUMAN MILK

Aleksandra Wesolowska[1], Kimberly Mansen[2], Kiersten Israel-Ballard[2], Maryanne Perrin[3]
[1]Medical University of Warsaw, [2]PATH, [3]University of North Carolina Greensboro

Donor human milk (DHM) is a critical part of preterm infant feeding therapies given evidence of protection against necrotizing enterocolitis compared to infant formula. An estimated 800,000 infants globally receive DHM each year, yet little is known about its nutritional composition or how it is influenced by milk banking processes and fortification. To address these knowledge gaps, we will examine and compare a broad range of nutrients in milk from 600 approved milk bank donors from the United States (US), India, Kenya, Poland, and Vietnam to create comprehensive, geographically diverse nutrient profiles for DHM. An overview of the National Institute of Health funded study protocol will be presented along with preliminary data from donors (n=200) from two US milk banks. Milk samples were assessed for true protein using the Kjeldahl method; for fat using the Mojonnier gravimetric method; and for lactose using the Megazyme enzymatic method which is not influenced by oligosaccharides. Most donors (177/200; 88.5%) gave birth at term. Mean lactation stage of donations was 20 weeks (range of 0 to 120 weeks); mean donation volume was 52 liters (range of 1 to 417). The mean macronutrient composition was: true protein 0.81 g/dL (95% CI 0.78 to 0.83); fat 3.3 g/dL (95% CI 3.2 to 3.4), lactose 6.7 g/dL (95% CI 6.7 to 6.8). The true protein values observed were significantly lower than clinical reference values for DHM in the United States (1.2 g/dL vs 0.8 g/dL) highlighting the need for reliable reference values. Fat composition was positively associated with lactation stage (standardized $\beta = 0.327$; $p = 0.0045$) and lactose was positively associated with lifetime donation volume (standardized $\beta = 0.233$; $p = 0.0458$). No nutrient differences were observed by maternal age or by gestation (term vs preterm). Differences in milk banking models globally may yield different findings.

OC 09

VEGAN/VEGETARIAN DIET AND HUMAN MILK DONATION: AN EMBA SURVEY ACROSS EUROPEAN MILK BANKS

Gandino Serena[1,2]*, Agnieszka Bzikowska[3], Aleksandra Wesolowska[3], Barbara Królak-Olejnik[3], Guido E. Moro[4], Enrico Bertino[1], Gillian Weaver[5]

[1]*Neonatal Care Unit of the University, City of Health and Science of Turin, Turin, Italy*

[2]*Nuffield Department of Women's & Reproductive Health, University of Oxford, John Radcliffe Hospital, Oxford (UK)*

[3]*Laboratory of Human Milk and Lactation Research, Regional Human Milk Bank of the Holy Family Hospital, Department of Medical Biology, Faculty of Health Sciences, Medical University of Warsaw, Warsaw, Poland*

[4]*Italian Association of Human Milk Banks (AIBLUD), Milan, Italy,*

[5]*The Human Milk Foundation, Hertfordshire, United Kingdom*

Background: extensive evidence demonstrates that human milk composition is related, in part, to maternal diet (therefore, to donors' diet in the case of donor human milk). Given the higher risk of nutritional deficiencies associated with plant-based diets (when not adequately planned and supplemented) concern has been raised about the nutritional adequacy of breast milk, when the mother follows a vegan/vegetarian diet. Very few studies have investigated the direct effect of a vegan/vegetarian diet on human milk. This has led to a variety of recommendations across Europe about the eligibility of vegan/vegetarian mothers to be recruited as human milk donors. As plant-based diets are gaining more and more popularity in Europe, it's therefore timely and important to explore this issue.

Objective: explore the attitude of European milk banks towards potential donors who follow a vegan or vegetarian diet, in order to develop common, expert-based recommendations on this topic.

Study design: Observational cross-sectional.

Methods: a web-based questionnaire was developed by the European Milk Bank Association (EMBA) "Maternal diet and donor human milk" working group, therefore distributed to the European human milk banks (HMBs). The questionnaire included 10 questions investigating the attitude of human milk banks towards potential donors following a vegan or vegetarian diet. Each human milk bank was requested to provide one reply only.

Results: preliminary data will be presented. 54 replies (response rate = 31%) have been received from 21 countries. Great variability in the attitude towards potential donors following a vegan or vegetarian diet has been detected across European milk banks, with only a minor proportion (30%) of HMB including vegan donors when taking adequate supplementations, while a consistent fraction (26%) excluded them consistently and another (37%) included them regardless of supplementation.

Conclusions: given the risk of nutritional deficiencies associated with vegan and vegetarian diets when not adequately supplemented and given the risk of severe clinical outcomes in infants breastfed by mothers following vegetarian/vegan diet, it is crucial to recommend adequate nutrient supplementations in potential donors following these restrictive diets, for the benefit of both infants receiving donor human milk, and infants exclusively breastfed by these cohorts. Common, European, expert-based recommendations on this topic are needed. Human milk banks can play a crucial health-promotion role by providing adequate nutritional counselling to breastfeeding mothers.

OC 10

MOTHER'S MILK IN CHRONIC KIDNEY DISEASE: A CASE-CONTROL STUDY

Gandino Serena[1,4]*, Tomasi Cont Alice[2], Botta Giulia[1], Gazzani Isabella[2], Antoniazzi Sara[3], Cavallarin Laura[3], Tonetto Paola[1], Attini Rossella[2], Giribaldi Marzia[3]
[1]Neonatal Care Unit of the University, City of Health and Science of Turin, Italy
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[3]Institute of Sciences of Food Production (ISPA) – CNR – Grugliasco (To), Italy
[4]Nuffield Department of Women's & Reproductive Health, University of Oxford, John Radcliffe Hospital, Oxford (UK)

Background: Given the lack of evidence regarding how maternal Chronic Kidney Disease (CKD) affects human milk composition, many clinicians prefer to apply a "precautionary approach" and discourage breastfeeding in mothers affected by renal failure, even in the absence of an official contraindication.
Objective: Evaluate milk composition in mothers affected by CKD and its nutritional adequacy.
Study design: Observational case-control study.
Methods: Pregnant women affected by nephrotic syndrome or CKD stage ≥ 3, followed-on in the Chronic Kidney Disease referral center of the Obstetrics and Gynecology Department, City of Health and Science of Turin, were recruited from February 2021 onward (n = 6). Each case was paired with 2 healthy controls, matched for gestational age at delivery (+/- 3 days), recruited in the Obstetrics and Gynecology Department, City of Health and Science of Turin. Milk was collected on the 7th-14th-28th-60th day post-delivery, stored at -20° and transferred to ISPA laboratories, Grugliasco, for analysis. Quantification and characterization of protein and non-protein nitrogen content was performed by using Dumas method and OPA method (Church, 1983), respectively. Auxological and neurodevelopmental follow-up of infants from mothers affected by CKD was performed at 40 weeks and 3 months of corrected age, in the Neonatal Care Unit of the University of Turin, by measuring weight, length, head circumference and by administering Brazelton Neonatal Behavioural Assessment Scale (NBAS). Linear mixed models' analyses will be conducted to compare cases' and controls' milk content.
Results: at present, 5 cases and 8 controls have been recruited from February 2021. Milk samples from each patient enrolled have been collected at 7th-14th-28th-60th day post-delivery, except for one 7-day-case, one 28-day-case and one 60-day-case samples missing. Of the 5 infants-cases, 2 received exclusive breastfeeding, 3 predominant breastfeeding. Growth and neurodevelopment follow-up of all 5 infants-cases have been performed at 40 weeks and 3 months of corrected age. A preliminary comparison of milk nitrogen content between cases and controls showed non-significant differences in case of well-controlled kidney disease. Although, the milk non-protein nitrogen content was higher in one case that presented higher blood urea in the two months post-delivery. Infants' follow-up showed an appropriate growth and neurobehavioral development in all the 5 studied cases.
Acknowledgement: the present study was supported by Fondazione CRT- Cassa di Risparmio di Torino (Mo.Mi.C.K. project).

POSTERS

P01

A BRIEF OVERVIEW OF THE FUNCTIONING OF THE FIRST SERBIAN HUMAN MILK BANK DURING THE COVID-19 PANDEMIC

R. Mileusnic-Milenovic, Z. Jelic
Institute of Neonatology, Belgrade

Aim: Analysis of the functioning of the First Serbian Human Milk Bank during the COVID-19 pandemic.

Method: Retrospective analysis of documentation from March 1, 2020 to April 1, 2022. We analyzed the method of work, the number of donors and the amount of collected milk.

Results: Due to the COVID-19 pandemic, the Department for Mothers and Child Nutrition (the milk bank is a part of this department) went through different phases. In April 2020 the department was closed for the reception of mothers and donor milk and one part of it was turned into the COVID-19 Department. During that time, we used milk from the stock in the bank. The department reopened at the end of September 2020 and is no longer closed but, to this day, it operates in a restrictive regime: the number of hospitalized mothers, from whom donors are recruited, is smaller. In the examined period we had a total of 779 L of milk: in 2020 y 213 L, in 2021 y 517 L and in the first three months of 2022 y 49 L. One mother, a donor for the second time, donated 400 L of milk in the analyzed period and she is the largest donor in the history of the bank. There have been no additional activities since the beginning of the pandemic.

Conclusion: Despite the most difficult period the bank has gone through since its foundation in 2009, it has maintained and continued to deliver donor milk without interruption due to the existence of stocks in the bank. The quantities of milk collected by the bank, except for the first year of the pandemic, were close to the quantities in regular circumstances thanks primarily to one donor who donated almost a year-round amount of milk to the bank.

P02

A PRELIMINARY STUDY ON BACTERIAL DIVERSITY OF HUMAN MILK: ASSOCIATION WITH MATERNAL FACTORS

A. Bzikowska-Jura, A. Koryszewska-Baginska, A. Wesolowska
Laboratory of Human Milk and Lactation Research at Regional Human Milk Bank in Holy Family Hospital, Department of Medical Biology, Medical University of Warsaw

The origin of human milk (HM) microbiota likely includes mother's skin, infant's mouth, and the transfer from maternal gastrointestinal tract.
The HM microbiome is highly variable and influenced by many factors, including those related to mother. Thus, the aim of our study was to characterize the microbiota of HM in healthy, exclusively breastfeeding Polish mothers and to investigate the effects of maternal factors (e.g age, anthropometric parameters, diet) on its diversity. HM samples from 15 women were collected within the 4-8 weeks postpartum.
All women were asked to provide HM samples in four time periods (6.00–12.00; 12.00-18.00; 18.00-24.00). At each time point, 5–10 mL of pre- and post-feed samples were obtained manually from the breast(s) that the infant fed from. HM microbiota profiling was analyzed by 16S rRNA gene sequencing, using the MiSeq sequencer (Illumina). To assess the amount of the obtained matrix, measurements were also made with the Qubit 3.0 fluorimeter (Thermo Scientific) and the dedicated Qubit High Sensitivity DNA kit reagent. Maternal dietary information was recorded through a food frequency questionnaire (FFQ) and 3-day dietary record, then clinical characteristics, including mode of delivery, socio-demographic data, and anthropometric measurements, were collected. Proteobacteria was the most abundant phylum, followed by Firmicutes and Bacteroidetes. Accordingly, Streptococcus was the most abundant genus. We did not reveal any correlations between maternal age and other socio-demographic data with microbiota variation. However, we observed that maternal fiber and vitamin C intake was potentially associated with Proteobacteria abundance ($p=0.039$). In turn, Lactobacillus abundance was positively correlated with vegetables and fruits intake. Additionally, mothers with higher pre-pregnancy BMI had a significantly higher abundance of Staphylococcus. Considering the crucial role of HM microbiome in shaping infant's immunity, further studies to clarify the potential impact of these maternal factors on HM bacterial diversity are needed.

P03

GESTATIONAL HYPERTENSION AND THEIR IMPACT ON ACTIVIN A CONCENTRATION OF THE HUMAN MILK

S. Sottemano[1], C. Peila[1], E. Spada[1], I. Barbagallo[2], G. Livoti[2], F. Galvano[2], D. Gazzolo[3]
[1]*Neonatal Care Unit of the University, City of Health and Science, Turin, Italy*
[2]*Department of Biological Chemistry, Medical Chemistry and Molecular Biology, University of Catania, Catania, Italy*
[3]*Neonatal Intensive Care Unit, Università degli Studi G. d'Annunzio Chieti e Pescara, Chieti, Italy*

Introduction: It is known that Preeclampsia affects the lactogenesis, but literature data on the effects of this syndrome on the neurobiomarkers composition and Activin A of Human Milk of the lactating mother are not available. The aim of this study is to investigate the effects of this gestational pathology on Activin A levels, a neurobiomarker known to play an important role in the development and protection of the central nervous system.
Methods: The women recruited in the study were divided in two different study groups: preeclamptic or healthy women. All the breast milk samples were collected using the same procedure into sterile devices BPA-free. Activin A was quantified using an ELISA test. To investigate the effect of pathology in the Activin a concentration in the 3 phases, mixed linear model with unistructural covariance structure, mother as random effect, and fixed effects were performed.
Results: Activin A was detected in all samples. There were no significant differences between Preeclamptic mother and Normotensive women. The only significant effect is related to the HM phase: in particular, it is significant the difference between colostrum and mature milk ($p<0.01$).
Conclusion: There is not any significant difference in Activin A breast milk composition from hypertensive and normotensive women. This result allows us to affirm that breast milk beneficial properties are maintained even if gestational hypertension occurs.

P04

HUMAN MILK PROTEIN OXIDATION: EFFECT OF HIGH PRESSURE PROCESSING

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Currently, pasteurized human milk is for premature babies. The pasteurization treatment can alter the nutritional properties of the milk. In order to reduce this impact of the stabilization treatment on the nutritional qualities of human milk, the High Pressure treatment could be an innovative alternative.The High Pressure treatment leads to the stabilization of food at an industrial scale, by inactivating microorganisms without a temperature rise. It uses pressure (200 to 600 MPa) and time (5 to 10 minutes) at low temperature (10-20°C). Human milk banks use the Holder pasteurization method (62.5°C for 30 minutes). Several studies have shown an increase in protein oxidation (rated by the amount of carbonylated proteins) following this pasteurization. Consequently, the oxidation mechanism is likely to alter the proteins of human milk, by modifying their structure and their functions.To evaluate the impact of the High Pressure treatment on the amount of oxidation of human milk proteins, an assay of carbonylated proteins with 2,4-dinitrophenylhydrazine (DNPH) was carried out on samples of skimmed milk. We compared fresh milk (control) with pasteurized milk (Holder) and milk treated by High Pressure. The High Pressure treatments applied were from 200 to 600 MPa, 5 minutes at 15°C. The content of carbonylated proteins in pasteurized human milk is significantly higher than that of raw milk, and milk treated by High Pressure. Treatments at 400 and 600 MPa cause much lower protein oxidation than Holder pasteurization. We previously obtained the same result with certain hormones.The High Pressure treatment seems to reduce the protein alteration, in comparison with the Holder pasteurization. Premature babies are very exposed to oxidative stress (infections, mechanical ventilation, perfusion) since their immune system is not mature. A decrease of the oxidation during the stabilization treatment of human milk could improve the protein bioavailability.

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HUMAN MILK-DERIVED MESENCHYMAL STEM CELLS AND GESTATIONAL DIABETES MELLITUS: EXPRESSION OF GLUCOSE TRANSPORTERS GLUT-1 AND GLUT-4

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INTRODUCTION: Several studies demonstrated that Mesenchymal Stem Cells (MSC) transplantation is an effective treatment in patients with Diabetes Mellitus since MSCs restore glucose blood concentration modulating Glucose Transporters (GLUTs). Recently, breast milk was identified as a source of stem cells such as MSCs. However, nothing is known about the contribution of Milk Derived MSC (MDMSC) on breast milk-induced glucose tolerance in the newborn and about MDMSCs GLUTs expression levels. Herein, we characterized MDMSCs phenotype and tested GLUT-1 and GLUT-4, the two main GLUTs members, gene expression.
METHODS: Milk samples were collected from physiological pregnant women and women with GDM pregnancies. After isolation, MDMSCs were characterized by flow cytometry assessing the expressions of CD105, CD90, CD73 and HLA-DR. Finally, MDMSCs Oct-4, NANOG, GLUT-1 and GLUT-4 gene expression levels were assessed by Real Time PCR.
RESULTS:All MDMSCs lines were positive for CD105, CD90 and CD73 surface antigens and negative for HLA-DR. Moreover, all MDMSCs lines properly expressed both stemness markers Oct4 and Nanog. Finally, GLUT-1 and GLUT-4 gene expression levels were down-regulated in GDM relative to control MDMSCs.
CONCLUSIONS: GLUTs downregulation in MDMSC from GDM pregnancies suggested a possible cellular compensatory response to maternal hyperglycemia in order to avoid newborn's disorders later in life.

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HUMAN MILK-DERIVED MESENCHYMAL STROMAL CELLS: FROM BENCH TO CLINICAL APPLICATION – A RESEARCH PROPOSAL

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Human Breast Milk (hBM) was recently identified as a source of Mesenchymal Stromal Cells (MSCs), a unique cellular population with renowned anti-inflammatory and immunemodulatory properties. Our recent data demonstrated that human Milk-derived MSCs (hMdMSCs) are characterized by the expression of neuromodulatory molecules as Brain-Derived Neurotrophic Factor (BDNF) and Neurotrophin 4 (NT4), immunemodulatory factors as Indoleamine-2,3-Dioxygenases 1 (IDO-1), Tumor Necrosis Factor-a (TNF-a) and Osteopontin (OPN) and glucose transporters GLUT-1 and GLUT-4. Importantly, we found these molecule more expressed in hMdMSCs isolated from the colostrum compared to transitional milk. Given these promising results, it looks clear that hMdMSCs could play a pivotal role in neonatal physiological development and, in particular, they could be of great benefit for those premature babies that do not have the opportunity to be feeded with fresh milk from their own mothers and require pasteurized donor milk. Since hMdMSCs do not express HLA-DR as we recently reported, they could be safely used as a heterologous treatment, for example to enrich pasteurized donor milk where the cellular component has been denatured by high temperature treatment.
Herein, we propose the development of a multicentric prospective research program aimed at standardizing isolation and characterization protocols, implementing GMP cultures production and defining the regulatory roadmap in order to bring hMdMSCs from bench to clinical use as cellular enrichment for donated human milk for premature babies.

P07

MAINTAINING HUMAN MILK BANK SERVICES THROUGHOUT THE COVID-19 PANDEMIC IN LITHUANIAN UNIVERSITY OF HEALTH SCIENCES KAUNAS CLINICS DHMB

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Introduction

The COVID-19 pandemic has presented a range of challenges to HMBs worldwide. Recent published data on viral infectivity from samples of women with confirmed COVID-19 have confirmed that there is no evidence that SARS-CoV-2 can be transmitted via breastmilk, supporting epidemiological evidence that there is minimal evidence of breastfeeding being a route of vertical transmission. However, HMB are facing considerable challenges during the pandemic in maintaining the operation of services, alongside uncertainty in terms of which additional practices, if any, should be introduced into HMB processes to maintain safety[1].

Experience of our HMB during COVID-19 pandemic The aim of our HMB is to collect human donor milk, store it and supply for the most vulnerable newborns. In order to keep high quality standards we are following NICE and Swedish HMB guidelines. During COVID-19 pandemic all mothers were screened prior to admission for COVID-19 symptoms and had PCR tests. Donor mothers were getting additional COVID-19 screening questions at the point of recruitment. We wanted to compare COVID-19 pandemic period (2020-2021) with pre-COVID period (2018-2019) in terms of number of donors, pasteurizations, amount of pasteurized donor breast milk and number of fed infants. We were happy to confirm that donor and pasteurization numbers remained similar and the number of newborns fed with donor breast milk slightly increased.

Discussion

The ongoing pandemic has impacted the functioning of HMB throughout the world [2]. The COVID-19 response to prevent infection and reduce global spread must also ensure that inadvertent harm is not done to other critical aspects of care and prevention.

COVID-19 has presented challenges and opportunities for health systems. However we have been able to maintain the safe operation of the HMB and meet the demand for donor breast milk.

P08

MILK EXPRESSION HABITS IN MATERNAL MILK DONORS; SHOULD WE FAVOR HINDMILK FOR BANKING?

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Background: A maternal milk bank was established in 2014 in Québec (Canada). The caloric contents of pooled milk lots (from ~4-8 mothers) ranges from 58-73 calories/100 ml, whereas most babies need ≥82 calories/100 ml. Hindmilk is 2-3 times fatter than foremilk but is only released 2-3 minutes post-letdown, so that the milk expression habits of donating mothers may influence caloric content. These habits are ill-documented, and precise recommendations on milk expression habits are needed.

Methods: An electronic questionnaire was emailed to all active milk donors in Québec to collect data on their milk expression habits and their willingness to change them.

Results/Findings: In total, 126 out of 181 donors (69.6%) completed the questionnaire. Most participants (77.7%) had a university degree, and 65.0% had >1 pregnancy. Overall, 97.6% were comfortable or very comfortable with current recommendations on milk expression, and 90.5% were aware or well aware of the benefits of maternal milk for preterm babies. However, 49.2% reported giving 0-2 times/week.

Concerning breastfeeding habits, 68.3% reported expressing milk for banking between breastfeedings; 17.5% reported expressing milk for banking from one breast while keeping the milk from their other breast for their baby; and 14.3% reported breastfeeding their baby on each breast and keeping hindmilk for banking. Overall, 65.8% of participants said they would be willing to change their habits most or all the time. Few participants commented on the complexity of milk expression or reported that adding further restrictions might discourage them from giving.

Conclusion: Most participating mothers were already giving foremilk and hindmilk. A significant proportion of them said would agree to change their expression habits in favor of giving hindmilk only. More information is needed on how changing recommendations for milk expression might impact the supply and caloric content of mothers' milk.

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POLYCHLORINATED BIPHENYLS IN BREAST MILK FROM POLISH WOMEN

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The presence of environmental pollutants is very dangerous for living organisms as these impurities can cause significant health problems.
Polychlorinated biphenyls (PCBs), which belong to the group of halogenated aromatic hydrocarbons, has been determined in breast milk.
Prenatal and postnatal exposure of infants to PCBs can be associated with health issues. Therefore, it was necessary to develop and adapt an analytical method to analyze PCB compounds.
The whole procedure was applied to 31 breast milk samples, which were collected from Polish mothers.
The QuEChERS method was optimized as a fast and cheap sample preparation method. The procedure allowed to obtain recovery values between 96.46% and 119.98% with acceptable relative standard deviations (3.36–12.71%). Gas chromatography with mass spectrometry (GC-MS) was used for final determination.
The mean concentration of #iPCBs in this study was 30.94 ng/g of lipid. Assigned daily intake of PCBs was lower than the tolerable daily intake, which shows that the analyzed milk is safe to the infants. However, the monitoring of PCBs in milk is still important, and the QuEChERS method with GC-MS can be an effective tool for tracking organic impurities in breast milk.

P10

PREEMIE®, A PORTABLE NIR SPECTROMETER FOR TARGETED FORTIFICATION IN NICU: PRELIMINARY RESULTS OF A PILOT STUDY

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The optimization of nutrition strategies in preterm infants plays a key role in determining the best possible developmental outcome. To this end, it is widely agreed upon that breast milk represents the best source of nutrition for all infants, even more so if born preterm. Due to the high nutritional demands of preterm newborns in the first months of life, it is essential to provide adequate fortification of breast milk.
To date, targeted fortification, an individualized fortification strategy, is the best fortification strategy available, as it adapts fortification to specific nutritional characteristics of that milk sample. Unfortunately its clinical application is severely limited by the need to use specific instrumentation, an infrared spectroscope.
Preemie® is a portable NIR spectroscope capable of measuring the composition of human milk in real time. The present study aims to participate in the development phases and validate a NIR spectrometer for bedside targeted fortification in NICU. 461 milk samples were acquired from healthy mothers of preterm and term infants, enrolled at the Neonatal Intensive Care Unit (NICU) of the University of Turin, S.Anna Hospital and the Milk Bank of Turin. Each sample, duly aliquoted and prepared, was analyzed by the Preemie® system for the measurement of the total levels of proteins, lipids and total energy. The data from Preemie® were compared with the values measured by laboratory analysis (gold standard) of the same samples. According to the preliminary results of the study, Preemie is currently able to analyze lipid and total energy values with <10% error. Calibration curves and training of Preemie's software for protein analysis are still under development.
The preliminary results of our study allow us to consider Preemie a tool to perform bedside targeted fortification of human milk, both maternal and donated, in a rapid and cost-effective manner.

P11

THE IMPORTANCE OF BREASTFEEDING DURING COVID 19 PANDEMY: MILK DONOR BANK SUPPORTS AND KEEP DONORS

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The human donor milk bank (HDMB) in Udine from 1966, uphold the goodness (generosity) and dedication of mothers that offer their milk to newborns that temporarily need, even in pandemic time.Nationwide, during the pandemic restrictions, number of donors decreased and amount of milk decreased too, despite after 2016 there were an increase in recruitment of mothers who decided to donate their milk.Udine's HDMB instead, during 2020, kept a steady number of donations, in contrast with the national trend: 60 mothers could store and offer their milk. We treated about 1700 liters of milk; 550 of them have been donated.Surely the avaibility of donated milk in our reality is the result of many factors: to name the most significant, the widespread diffusion of breastfeeding courses aimed to dedicated staff, the information addressed to pregnant women about the importance of breastfeeding, even for mothers Covid positive, who staied safetly in a room with their newborns (24/24 hours rooming in), thanks to the attentive presence of nurses that encorauged breast attack promoting breast feeding.Indeed few newborns (children) from symptomatic sick mothers were separated from them; emblemathic is the instance of a mother of two twins that, even if she could see her babies (late preterms) only by video call, she kept on nourishing them pumping her milk.

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ANUE CONCEPT™# NEW AND UNIQUE COLLECT AND STORAGE SYSTEM

J. Grønnslett
Anue, Bodø, Norway

Current methods for the storage and transport of breastmilk involve the use of simple containers such as plastic pouches, bottles, or zip lock bags. Typical state of the art products have numerous disadvantages with regards to long-term storage, transport and testing. These disadvantages are particularly apparent in the field of donor breastmilk. Donor breastmilk is typically collected at the home of the donor, where it is frozen for longer-term storage, or for later transportation (e.g. to a donor milk bank or hospital). Typically the breastmilk must be thawed at the hospital before a sample can be taken for testing. However, during collection and testing, the breastmilk is susceptible to contamination. As a result, current methods are lacking when it comes to collection and transportation of frozen breastmilk.
Anue Concept™# is a new and unique,patent pending, collect and storage system that make it possible to have a representative milksample for testing without risk of contaminating or thawing the milk. It will provide a close system for the milk all the way from donor to child. The Concept have been testet and developed togheter with the Milk Bank in Nordland Hospital and Oslo University Hospital in Norway.
The purpose is to provide safe human donor milk with better quality, taste and durability.

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