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Mapping the current knowledge on leukocytes in human breastmilk: a scoping review protocol

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Abstract

INTRODUCTION

The immunological composition of breastmilk has gained research interest as breastfeeding has persistently correlated with improved health outcomes in the child. Immune cells, also known as leukocytes, are key components of the body's immune system, but they remain understudied in breastmilk. The relevance of breastmilk leukocytes for breastfeeding-mediated immune benefits remains controversial. To identify the current State-of-the-Art on breastmilk leukocyte research, unearth knowledge gaps, and propose research priorities, a scoping review is necessary.

METHODS AND ANALYSIS

This scoping review will address the general question of what is known about leukocytes in human breastmilk. The development of this scoping review protocol adhered to the recommendations set forth by the Joanna Briggs Institute guidelines. Peer-reviewed research articles published in English, French, or Spanish will be eligible for inclusion in the scoping review. The initial literature search was conducted in January 2024 within the Medline, Embase, Cochrane central, and BVS databases.

ETHICS AND DISSEMINATION

This review does not require ethics approval. Our dissemination strategy includes peer review publication, presentations at conferences and to relevant stakeholders.

REGISTRATION DETAILS

This protocol was registered in Open Science Framework, (osf.io/kwfsy) on February 19th, 2024.

ARTICLE SUMMARY

Strengths and limitations of this study

- This scoping review represents the first systematic exploration of the literature on leukocytes in human breastmilk, underscoring the novelty of this research.
- We propose a novel system for quality appraisal of the literature on qualitative and quantitative characterization of breastmilk leukocytes.
- We will focus our analysis on peer-reviewed literature only, deposited in 4 databases, and published either in English, French or Spanish. Therefore, some relevant publications indexed in other places or in a different language may be missed.

Keywords: Colostrum, leukocytes, breastfeeding, breastmilk, immune cells, human milk

Introduction

Breastfeeding is encouraged by multiple agencies worldwide to promote health in both infants and mothers. Beyond aiding development through nutrition, breastmilk provides maternally-derived immune factors to suckling infants, including antibodies, cytokines, and growth factors[1–3]. These components protect infants from infectious diseases, sustain the maturation of the intestine, and promote the establishment of the commensal microbiota [4,5].

In addition to soluble factors, live immune cells, also called leukocytes, have been found in breastmilk. The composition of breastmilk leukocytes (BreLeuk) is dynamic and varies together with mother and infant health, maternal BMI, and gestational age at birth, among other conditions impacting the mother-infant dyad [5–7]. Along the process of lactation, from the development of the mammary glands until involution, leukocytes of multiple lineages including lymphocytes, granulocytes, monocytes, and macrophages travel from peripheral tissues to the maturating mammary gland by blood and lymph, reside *in situ* and are found in breastmilk [8–10]. The exact process regulating the presence of leukocytes in breastmilk remains elusive.

A variety of functions or roles have been proposed for BreLeuk. For instance, B lymphocytes produce the antibodies responsible for the passive immunity provided by breastmilk. While antibodies have been shown to travel through the transcytosis route from mammary tissue into breastmilk, antibody-producing B lymphocytes have also been described in breastmilk [11,12]. In mice, BreLeuk could survive the digestion process and migrate to various tissues from suckling pups, effectively creating microchimerism [13,14]. Additional animal studies have described a specific T lymphocyte subpopulation that may be transferred exclusively from breastmilk through multiple generations, and that significantly impacts local immunity and the intestinal microbiota [15]. Unique lactation-induced macrophages were recently described in mouse breastmilk to also impact the microbiota establishment [16]. BreLeuk could directly participate in microbiota and pathogen regulation *in situ* through their multiple functions, for example phagocytosis [5].

Therefore, scattered information has documented BreLeuk in a variety of contexts, and BreLeuk may play relevant roles directly in the milk within the mammary gland, or once ingested in the infant's intestine. Available reports are not comprehensive, focusing on a limited number of leukocyte lineages at a time, and/or maternal-infant conditions. Additionally, a limited number of studies pertain to the human species. The heterogeneity of breastmilk composition during lactation further complicates painting a comprehensive picture of the current landscape in human BreLeuk science [8]. Given the probable relevance of BreLeuk for promoting health in suckling infants, we propose here a protocol for a scoping review to systematically review the scientific literature reporting on human BreLeuk.

A search in PROSPERO, Open Science Framework, MEDLINE, Embase, Cochrane Database of Systematic Reviews and BVS was conducted, and no review on this topic was found the time of writing (January 2024). The objective of this scoping review is to identify and organize the available information from the peer-reviewed original scientific literature about maternal leukocytes (including but not restricted to proportions,

concentrations, subsets and described functions) in human colostrum/transitional and mature milk.

Methods and analysis

Study design

The aim of this protocol is to answer the research question formulated as:
"*What is known about human breastmilk leukocytes present in human milk during lactation?"*

The purpose of this review is to synthesize relevant qualitative and quantitative data from experimental research studies reporting on human BreLeuk. We will select peer reviewed research articles containing information on any human BreLeuk lineage and report the information together with its associated metadata. The proposed scoping review protocol has been developed following the JBI guidance for scoping reviews [17], and has been registered with Open Science Framework (https://osf.io/kwfsy). In addition, this protocol complies with the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) Statement for protocols, for all items that apply to scoping reviews (**Supplementary table 1**) [18]. We formulated the search strategy based on our research question, following the Population, Concept, and Context (PCC) framework as shown in **Table 1.**

Sources

The 4 selected databases for articles retrieval are Medline (OVID), Embase, Cochrane Central Register of Control Trials and BVS.

The relevant studies considered for this review will exclusively be peer-reviewed, published primary research articles. Study designs eligible encompass experimental and quasi-experimental, including randomized and non-randomized controlled trials, and analytical observational studies (prospective case-control studies and analytical cross-sectional studies). Case series, individual case reports and descriptive cross-sectional studies are also eligible. Excluded studies include opinions, reviews, gray literature, conference abstracts, and books. Articles included in the final output must satisfy the criteria described in **Table 2.** If a relevant article is not freely available online, support will be sought from the participants' institutional library services and by contacting the article's corresponding author(s).

Search strategy

On January 25^{th,} 2024, an exploratory search was undertaken by an expert librarian (S.D.), restricted to the Medline database. The strategy involved applying keywords, and medical subject headings (MeSH) terms that associate to relevant key concepts, together with Boolean terms “AND” and “OR”. The MeSH terms used were the following: "Milk, Human", "Lactation", "Breast Feeding", "Colostrum", "Leukocytes", "Lymphocytes", "Killer Cells, Natural", "Monocytes", "Dendritic Cells", "Granulocytes", "Neutrophils", "Eosinophils", "Basophils", "Natural Killer T-Cells", "B-Lymphocytes". The free terms used for this initial search were: Human milk, mature milk, breast milk, Maternal milk, transitional milk, Foremilk, Hindmilk, breastmilk, breastfeed*, "Breast feed*", Maternal transfer, Colostr*, Lactation, leukocyte*, lymphocyte*, B-Lymphocytes, myeloid, lymphoid, "Natural Killer", "NK Cells", Monocytes, Macrophage*, "dendritic cell*", "White blood cells", "White blood Corpuscle", "Immun* cells", Granulocytes, Neutrophil*,

Eosinophil*, Basophil, "B cells", "T cells". The search was restricted to the human species; however, the exploratory search retrieved a very large proportion of non-human animal studies prompting iterations to the search strategy. Nine known relevant publications proposed by BreLeuk expert team members were confirmed to be present in the search output, which validated its scope.

The final search was performed on January 31st, 2024 in MEDLINE using the search string detailed in **Supplementary Appendix 2.** The strategy was then adapted by the expert librarian to the 3 additional databases (**Supplementary Appendices 3-5).**

Selection of sources of evidence

A total of 4193 articles were originally retrieved from the 4 databases and loaded to Rayyan platform [19], where 291 duplicates were deleted, giving a final selection of 3953 articles available for screening. The first screening process will be conducted blindly and independently by 3 researchers from the team (B.J., C.G., E.S.), using Rayyan. Screening will involve scrutiny of titles and abstracts of each article according to the selection criteria described in **Table 2**. Discrepancies in screening will be resolved by consensus after a thorough revision involving an additional topic expert from the team (M.B.). The second screening will be based on the full text version of each article to confirm relevance and will be performed by 3 team members (B.J., C.G., E.S.), independently. A preliminary flowchart of this process is described in **Figure 1**, that follows the Prisma-ScR guidelines [20].

Charting the data

Once the final list of articles to be included in this scoping review is complete, the full text versions of the articles will be scrutinized to collect relevant metadata. This will be done by 3 team members (B.J., C.G., and E.S.), independently, and the data will be charted automatically into an excel document using a Google form. Advantages of using a google form for the data charting process includes compulsory filling of information, leading to no empty fields that may cause confusion between unavailable data and the answer "No". It also allows the team to be working simultaneously, blindly and unambiguously on the same publication. The google form questionnaire elaborated to collect information for the scoping review is described in **Table 3.** While various questionnaire items may seem redundant (for example, Q24 and Q25), this will allow a more robust description of the studies (in the previous example, "colostrum" labelling has been used to describe breastmilk collected <2 days postpartum in some studies, but until <7 days postpartum in others [7,21–23]).

Analysis and presentation of results

We will adhere to the PRISMA-ScR guidelines to report the findings from this study [19]. The full list of included articles will be provided as part of the publication. The results of the scoping review will be categorized according to relevant themes including but not restricted to: study demographics, methodologies, and leukocyte subtype. These results will be presented as evidence maps, such as tables and diagrams, and narrated in text.

Metadata will be summarized to provide a comprehensive overview of the field, and specific relevant findings will be detailed, including frequency and concentration of leukocyte subtype in milk.

Sub-themes regarding the heterogeneity of populations (demographics), or maternal health status may emerge and will be described and discussed, as relevant.

Evidence quality appraisal

We anticipate the retrieved articles to be mostly observational studies. Given the unavailability of a quality assessment tool for the data in our scoping review, we developed a tool based on the JBI Critical Appraisal Checklist [24], merged with the adaptation of the Cochrane risk of bias tool, done by Wylde and colleagues in 2017 [25] . Our tool will grade each included paper for risk of bias, from 0 (no identified risk) to 1 (low risk, minor inconsistencies) to 2 (high risk, major inconsistencies), in answering the 5 questions below:

1. Were the criteria for mother inclusion in the study clearly defined?
2. Were technical confounding factors identified? (e.g. viability marker not used for flow cytometry, etc.)
3. Were quantitative data provided for all analyzed leukocytes declared in methods?
4. Were the methods described in sufficient details to allow reproducible experiment?
5. Other bias

Discussion

Breastfeeding is a time-restricted opportunity to positively impact short- and long-term health. As research moves from empirical evidence to mechanistic explanations for the known benefits of breastmilk, BreLeuk emerges as enigmatic participants to breastmilk mediated health. The scoping review proposed in this protocol will help summarize what is known about leukocytes in human breastmilk, providing quantitative and qualitative evidence to help direct future research efforts.

Ethics and dissemination

This review does not require ethics approval. Our dissemination strategy includes peer review publication, presentations at conferences and to relevant stakeholders.

Footnotes

Availability of data and material

Additional data can be found in Supplementary materials.

Competing interests statement

The authors do not have any competing interest to declare.

Funding statement

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Author contributions

C.G. and B.J. produced the preliminary search strategy, wrote and edited the protocol and developed the data charting form. S.C.D., J.Y.N. and A.M.L. produced and edited the exploratory search and final search strategies, and produced and edited the manuscript. M.B. and E.S. conceived the study, wrote and edited the manuscript, and M.B additionally secured research funding. All authors approved the final version of the manuscript.

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Figure

Figure 1. PRISMA flow diagram.

Tables

Table 1. Population-Concept-Context

Population	Any human study reporting data from leukocytes in human milk will be included, irrespective of maternal demographic or clinical status.
Concepts	Literature reporting on leukocytes (including, but not limited to granulocytes, monocytes, macrophages, B and T lymphocytes, dendritic cells, natural killers, and their progenitors), their measurements and identification in all stages of human milk (colostrum, transitional and mature milk) will be included in the scoping review. Associated metadata reporting on maternal/infant demographics and clinical characteristics, sample collection, milk and cell processing for analysis will also be charted.
Context	This review will examine any reported scenarios of human milk analyzed for the proportions, counts, or phenotype of any leukocyte subpopulation. The geographical location, stages of milk maturation (colostrum, transition and mature milk), maternal or infant health status, or study designs will not be limited.

Table 2. Inclusion and Exclusion Criteria

	Inclusion	Exclusion
Article type	Published peer-reviewed research articles	Review articles, pre-print articles, conference abstracts, grey literature
Language	English, Spanish & French	All languages other than English, Spanish & French
Country	All countries	None
Publication date	From inception until January 31st, 2024	Studies published after January 31st, 2024
Model of research	Human	Other than human
Cell population of characterization/evaluation	Leukocytes, including, but not restricted to: • Neutrophils • Eosinophils • Basophils • Monocytes	Any other cell type present in breast milk including hematopoietic stem cells

	• Macrophages • B lymphocytes • T lymphocytes • Dendritic cells • Natural killers	
Milk sampling	Any type of human milk (colostrum, transitional milk or mature milk), at any time of sampling (pre- and post-feeding, mornings/evenings, etc).	No restriction
Demographic & clinical data of the cohort participants	Human mothers, mothers of all ages, nationalities, health stages. All types of delivery Any neonate sex. Any neonatal term (pre-term, full term, post-term Any maternal or infant condition)	No restriction
Extras	Maternal pathologies or special medical conditions	No restriction

Table 3. Metadata: charting items

Publication information
Q1. Complete publication title
Q2. Authors
Q3. Nomenclature of the article from our database
Q4. Language
Q5. Keywords used
Study design *Please describe the parameters of the study in more detail (e.g. follow-up length, intervention, etc...)*
Q6. What was the overall aim of this study
Q7. Study type Observational study, intervention, cross-sectional, longitudinal, retrospective, prospective and/or other)
Q8. Complete cohort size (number of mothers included in the study)

Q9. Subgroups description (e.g. obese vs. lean) and size
Q10. Patients' dropout (only in clinical trials) --> If not relevant: NA, if relevant, add number of dropouts
Q11. Are infants also considered/described in this work?

Demographic & clinical data of the cohort participants

Q12. Nationality of the mothers
Q13. Age range of mothers (e.g. 18-38)
Q14. Health status of the test group of mothers (preeclampsia, gestational diabetes, COVID-19, HIV and/or other)
Q15. Additional information about mothers? (Ethnicity, socio-economic background, BMI, recruitment from a special group, etc...)
Q16. Recorded maternal medications or supplements of any kind (even outside of an intervention)
Q17. If infants were considered, what specific health status are included? (Healthy, term, pre-term, infections and/ or other)
Q18. If infants with infections were considered, which pathogen(s) is/are involved?
Q19. If infants with non-infectious diseases were included, describe which ones
Q20. Delivery method (vaginal delivery or C-section)
Q21. Gestational age (if available, in weeks)
Q22. Maternal food intake? No or yes with details.
Q23. Number of delivery of the mother

Milk sampling information

Q24. Milk type description (colostrum, transitional milk, mature and/or other)
Q25. Timing of sample collection postpartum (in number of days)
Q26. Information on exact timing of collection within the day (e.g. morning, pre-or post-infant feeding, 30min into feeding, etc...)
Q27. Methodology for collection (manual vs. automatic pump, cleaning of the area, discard first drops, etc...)
Q28. Range of volumes collected. Or exact volume if the case may be
Q29. Processing method (e.g. length of time between collection and processing, length of processing post collection (could affect neutrophil measurements, leukocyte activation, etc..), dilutions (in what buffer/medium?), centrifugation speed? discard supernatant? enrichment of populations (by density gradient, magnetic beads, etc..?), temperature of processing)
Q30. Long-term storage temperature
Q31. Other bioactives measured from milk samples?
Q32. Any confoundable aspects in the methodology details?

Leukocyte analysis*

We have 8 defined questions (Q34-Q41) aimed to gather as much information as possible of each analyzed leukocyte. Reactives Q41-Q121 correspond to the repetition of these 8 questions, available for as many leukocyte subtypes reported in each publication.

Q33. All immune cell populations of interest in this study

Q34. Immune cell population of interest in this section (First)

Q35. Description of the regulation of the phenotype of that cell in paper

(expression of surface molecules, cytokines, modification of function)

Leukocyte #1

Q36. Type of analysis performed (flow cytometry, microscopy, transcriptomic analysis and/or other)

Q37. In the case of flow cytometry analyses, indicate what gating strategy was used to identify each population

Q38. In the case of flow cytometry specify all that apply: (Use/not use of viability marker, gating/not gating on single cells, use/not use of FMOs, gating strategy provided/not provided as a figure, clear/unclear parental population description)

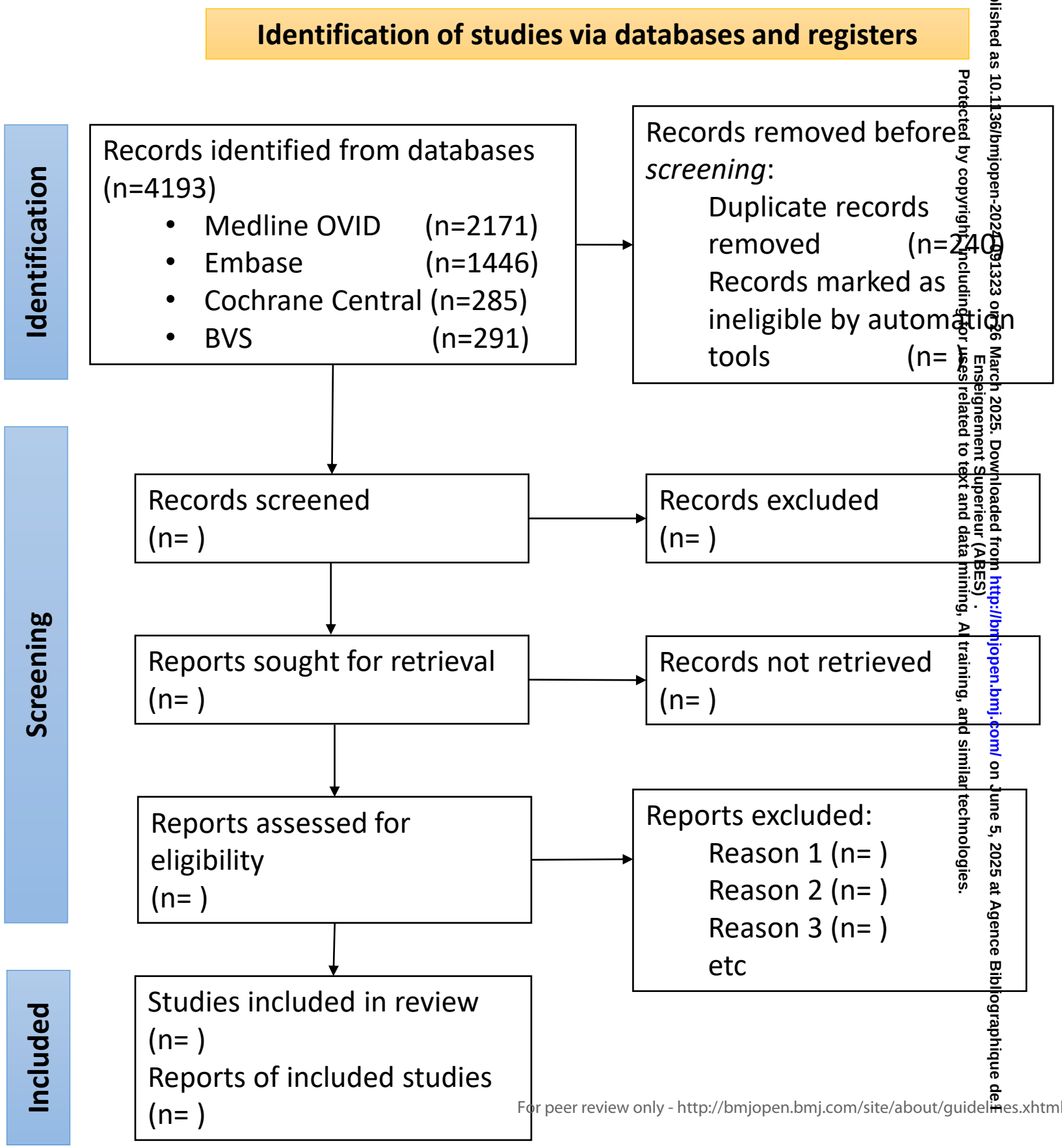
Q39. Additional methodology details

Q40. Methodology and results (Identity or phenotype modification (concentrations, phenotype, changes between groups/treatments, other relevant results on leukocytes in milk)

Q41. Additional information of interest outside of current categories

Q42-Q121. Repetition of the questions Q33 to Q41 for the different leukocytes that are described in the studies.

Figure 1



PRISMA-P 2015 Checklist for Scoping review protocol: Mapping the current knowledge on leukocytes in human breastmilk

This checklist has been adapted for use with protocol submissions to *Systematic Reviews* from Table 3 Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review **Our protocol is designed specifically to undertake a scoping review**	☒	☐	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	☒	NA
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	☒	☐	55-56
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	☒	☐	3-28
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	☒	☐	233-238
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	☐	☒	NA
Support					
Sources	5a	Indicate sources of financial or other support for the review	☒	☐	229-232
Sponsor	5b	Provide name for the review funder and/or sponsor	☐	☒	NA
Role of	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☐	☒	NA

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
sponsor/funder					
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	☒	☐	90-99
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO) **We used Population, Concepts, Context (PCC) instead**	☒	☐	108-110, 131-133
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review **Inclusion criteria was described according to PCC**	☒	☐	131-133
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	☒	☐	123-124
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	☒	☐	138-157
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	☒	☐	171-183
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	☒	☐	159-169
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	☒	☐	171-183
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☒	☐	Table 3
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	☐	☒	NA
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	☐	☒	NA

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☒	☐	186-189
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	☐	☒	NA
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☐	☒	NA
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	☒	☐	185-193
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☐	☒	NA
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☒	☐	195-203

Supplementary Appendix 2. Search strategy for Medline

Characteristic	Report
Type of search	New
Database	Medline
Platform	Ovid
Search date	03/02/2024
Date range for the search	No filter
Language restrictions	No filter
Other limitations	None
Search strategy	1. exp "Milk, Human"/ (23092) 2. exp "Lactation"/ (48800) 3. exp "Breast Feeding"/ (44731) 4. exp "Colostrum"/ (6930) 5. ((Human or mature or breast or Maternal or transitional) adj5 milk).tw. (28502) 6. Foremilk.tw. (244) 7. Hindmilk.tw. (85) 8. breastmilk.tw. (1721) 9. breastfeed*.tw. (29711) 10. "Breast feed*".tw. (13265) 11. (Maternal adj3 transfer).tw. (2152) 12. Colostr*.tw. (8455) 13. Lactation.tw. (37879) 14. or/1-13 (138862) 15. exp "Leukocytes"/ (830044) 16. exp "Lymphocytes"/ (579306) 17. exp "Killer Cells, Natural"/ (48402) 18. exp "Monocytes"/ (69247) 19. exp "Dendritic Cells"/ (54566) 20. exp "Granulocytes"/ (154790) 21. exp "Neutrophils"/ (97208) 22. exp "Eosinophils"/ (26104) 23. exp "Basophils"/ (8038) 24. exp "Natural Killer T-Cells"/ (3244) 25. exp "B-Lymphocytes"/ (104787) 26. leukocyte*.tw. (150835) 27. lymphocyte*.tw. (348191) 28. B-Lymphocytes.tw. (25520) 29. myeloid.tw. (109500) 30. lymphoid.tw. (85385) 31. ("Natural Killer" or "NK Cells").tw. (59121) 32. Monocytes.tw. (76287) 33. Macrophage*.tw. (289725) 34. "dendritic cell*".tw. (67588) 35. ("White blood" adj4 (cells or Corpuscle)).tw. (12908) 36. "Immun* cells".tw. (75475)

	37. Granulocytes.tw. (23427) 38. Neutrophil*.tw. (159175) 39. Eosinophil*.tw. (79470) 40. Basophil.tw. (5642) 41. "B cells".tw. (80857) 42. "T cells".tw. (254893) 43. or/15-42 (1526669) 44. 14 and 43 (4772) 45. 44 not ((animals.sh. or nonhuman.tw. or exp animals/) not humans.sh.) (2372) 46. (animals/ or Models, Animal/ or Disease Models, Animal/) not Humans/ (5155715) 47. ((animal or animals or canine* or cattle or bovine or buffalo or dog or dogs or feline or hamster* or lamb or lambs or mice or monkey* or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or cow* or veterinar*) not (human* or patient)).ti,kw,jw. (2464566) 48. 46 or 47 (5460647) 49. 45 not 48 (2171)
References	2171

Supplementary Appendix 3. Search strategy for Embase

Characteristic	Report
Type of search	New
Database	Embase
Platform	Embase.com
Search date	31/01/2024
Date range for the search	No filter
Language restrictions	No filter
Other limitations	None
Search strategy	1. 'breast milk'/exp (36631) 2. 'lactation'/exp (65746) 3. 'breast feeding'/exp (69736) 4. 'colostrum'/exp (10006) 5. ((human OR mature OR breast OR maternal OR transitional) NEAR/5 milk):ab,ti (38502) 6. foremilk:ab,ti (294) 7. hindmilk:ab,ti (127) 8. breastmilk:ab,ti (3888) 9. breastfeed*:ab,ti (52592) 10. 'breast feed*':ab,ti (17528) 11. (maternal NEAR/3 transfer):ab,ti (2894) 12. colostr*:ab,ti (10140) 13. lactation:ab,ti (49637) 14. #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 (189970)

15. 'leukocyte'/exp (1531736)
16. 'lymphocyte'/exp (1075920)
17. 'natural killer cell'/exp (102952)
18. 'monocyte'/exp (142898)
19. 'dendritic cell'/exp (130813)
20. 'granulocyte'/exp (273455)
21. 'neutrophil'/exp (179372)
22. 'eosinophil'/exp (63814)
23. 'basophil'/exp (17658)
24. 'natural killer t cell'/exp (13247)
25. 'b lymphocyte'/exp (224780)
26. leukocyte*:ab,ti (217318)
27. lymphocyte*:ab,ti (501699)
28. 'b lymphocytes':ab,ti (34133)
29. myeloid:ab,ti (199944)
30. lymphoid:ab,ti (124380)
31. 'natural killer':ab,ti OR 'nk cells':ab,ti (93279)
32. monocytes:ab,ti (117481)
33. macrophage*:ab,ti (416217)
34. 'dendritic cell*':ab,ti (106347)
35. ('white blood' NEAR/4 (cells OR corpuscle)):ab,ti (22717)
36. 'immun* cells':ab,ti (130081)
37. granulocytes:ab,ti (32973)
38. neutrophil:ab,ti (159042)
39. eosinophil*:ab,ti (130677)
40. basophil:ab,ti (9035)
41. 'b cells':ab,ti (126024)
42. 't cells':ab,ti (402796)
43. #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40 OR #41 OR #42 (2402009)
44. #14 AND #43 (7932)
45. #44 AND ('Conference Abstract'/it OR 'Conference Paper'/it OR 'Conference Review'/it) (1337)
46. (animal:ti,ab,lnk,kw,tn,tt,df,mn,dn OR animals:ti,ab,lnk,kw,tn,tt,df,mn,dn OR canine*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR cattle:ti,ab,lnk,kw,tn,tt,df,mn,dn OR bovine:ti,ab,lnk,kw,tn,tt,df,mn,dn OR buffalo:ti,ab,lnk,kw,tn,tt,df,mn,dn OR dog:ti,ab,lnk,kw,tn,tt,df,mn,dn OR dogs:ti,ab,lnk,kw,tn,tt,df,mn,dn OR feline:ti,ab,lnk,kw,tn,tt,df,mn,dn OR hamster*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR lamb:ti,ab,lnk,kw,tn,tt,df,mn,dn OR lambs:ti,ab,lnk,kw,tn,tt,df,mn,dn OR mice:ti,ab,lnk,kw,tn,tt,df,mn,dn OR monkey*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR mouse:ti,ab,lnk,kw,tn,tt,df,mn,dn OR murine:ti,ab,lnk,kw,tn,tt,df,mn,dn OR pig:ti,ab,lnk,kw,tn,tt,df,mn,dn OR pigs:ti,ab,lnk,kw,tn,tt,df,mn,dn OR piglet*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR porcine:ti,ab,lnk,kw,tn,tt,df,mn,dn OR primate*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR rabbit*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR rats:ti,ab,lnk,kw,tn,tt,df,mn,dn OR rat:ti,ab,lnk,kw,tn,tt,df,mn,dn OR rodent*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR sheep*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR cow*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR veterinar*:ti,ab,lnk,kw,tn,tt,df,mn,dn) NOT

	(human*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR patient:ti,ab,lnk,kw,tn,tt,df,mn,dn) (4569866) 47. #45 NOT #46 (973) 48. #44 NOT #45 (6595) 49. ('animal'/exp OR 'juvenile animal'/exp OR 'adult animal'/exp OR 'animal cell'/exp OR 'animal tissue'/exp OR 'nonhuman'/exp OR 'animal experiment'/exp OR 'animal model'/exp) NOT 'human'/exp (8024124) 50. #48 NOT (#46 OR #49) (3295) 51. #47 OR #50 (4268) 52. #51 AND [embase]/lim NOT ([embase]/lim AND [medline]/lim) (1446)
References	1446

Supplementary Appendix 4. Search strategy for Cochrane Central Register of Controlled Trials

Characteristic	Report
Type of search	New
Database	Cochrane Central Register of Controlled Trials
Platform	Ovid
Search date	31/01/2024
Date range for the search	No filter
Language restrictions	No filter
Other limitations	None
Search strategy	1. exp "Milk, Human"/ (1274) 2. exp "Lactation"/ (938) 3. exp "Breast Feeding"/ (2639) 4. exp "Colostrum"/ (216) 5. ((Human or mature or breast or Maternal or transitional) adj5 milk).tw. (4026) 6. Foremilk.tw. (8) 7. Hindmilk.tw. (16) 8. breastmilk.tw. (395) 9. breastfeed*.tw. (6571) 10. "Breast feed*".tw. (2833) 11. (Maternal adj3 transfer).tw. (69) 12. Colostr*.tw. (556) 13. Lactation.tw. (2862) 14. or/1-13 (14866) 15. exp "Leukocytes"/ (11224) 16. exp "Lymphocytes"/ (6319) 17. exp "Killer Cells, Natural"/ (878) 18. exp "Monocytes"/ (850) 19. exp "Dendritic Cells"/ (368) 20. exp "Granulocytes"/ (2844) 21. exp "Neutrophils"/ (1587) 22. exp "Eosinophils"/ (929) 23. exp "Basophils"/ (155)

	24. exp "Natural Killer T-Cells"/ (17) 25. exp "B-Lymphocytes"/ (634) 26. leukocyte*.tw. (6984) 27. lymphocyte*.tw. (13034) 28. B-Lymphocytes.tw. (431) 29. myeloid.tw. (6405) 30. lymphoid.tw. (1172) 31. ("Natural Killer" or "NK Cells").tw. (2694) 32. Monocytes.tw. (2321) 33. Macrophage*.tw. (4471) 34. "dendritic cell*".tw. (1618) 35. ("White blood" adj4 (cells or Corpuscle)).tw. (1613) 36. "Immun* cells".tw. (1907) 37. Granulocytes.tw. (725) 38. Neutrophil*.tw. (12778) 39. Eosinophil*.tw. (5730) 40. Basophil.tw. (417) 41. "B cells".tw. (1883) 42. "T cells".tw. (6638) 43. or/15-42 (55958) 44. 14 and 43 (308) 45. 44 not ((animals.sh. or nonhuman.tw. or exp animals/) not humans.sh.) (300) 46. (animals/ or Models, Animal/ or Disease Models, Animal/) not Humans/ (2656) 47. ((animal or animals or canine* or cattle or bovine or buffalo or dog or dogs or feline or hamster* or lamb or lambs or mice or monkey* or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or cow* or veterinar*) not (human* or patient)).ti,kw,jw. (6393) 48. 46 or 47 (7313) 49. 45 not 48 (285)
References	285

Supplementary Appendix 5. Search strategy for BVS

Characteristic	Report
Type of search	New
Database	LILACS, WPRIM, LIS, IBECS, BDENF. CUMED, LIPECS, SES-SP, BBO, BRISA, VETINDEX, colecionaSUS and INDEXPSI
Platform	BVS
Search date	31/01/2024
Date range for the search	No filter
Language restrictions	No filter
Other limitations	None
Search strategy	(((lactation OR "breast milk" OR breastmilk OR "breast feed*" OR "leche materna" OR colostr* OR calostro OR lactancia OR amamanta*)) AND ((leucocitos OR leukocytes OR linfocitos OR lymphocytes OR "células

	asesinas naturales" OR "natural killer cells" OR monocitos OR monocytes OR "células dendríticas" OR "dendritic cells" OR granulocitos OR granulocytes OR neutrófilos OR neutrophils OR eosinófilos OR eosinophils OR basófilos OR basophils OR "células T asesinas naturales" OR "natural killer T cells" OR "linfocitos B" OR "B lymphocytes" OR mieloide OR myeloid OR linfoide OR lymphoid OR macrófagos OR macrophages OR "white blood" OR "células B" OR "B cells" OR "células T" OR "T cells"))) AND NOT (ti:((animal OR animals OR canine* OR cattle OR bovine OR buffalo OR dog OR dogs OR feline OR hamster* OR lamb OR lambs OR mice OR monkey* OR mouse OR murine OR pig OR pigs OR piglet* OR porcine OR primate* OR rabbit* OR rats OR rat OR rodent* OR sheep* OR cow* OR veterinar*))) AND (db:("LILACS" OR "WPRIM" OR "LIS" OR "IBECS" OR "BDENF" OR "CUMED" OR "LIPECS" OR "SES-SP" OR "BBO" OR "BRISA" OR "VETINDEX" OR "colecionaSUS" OR "INDEXPSI"))
References	291

BMJ Open

Mapping the current knowledge on leukocytes in human breastmilk: a scoping review protocol

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Mapping the current knowledge on leukocytes in human breastmilk: a scoping review protocol

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Abstract

INTRODUCTION

The immunological composition of breastmilk has gained research interest as breastfeeding has persistently correlated with improved health outcomes in the child. Immune cells, also known as leukocytes, are key components of the body's immune system, but they remain understudied in breastmilk. The relevance of breastmilk leukocytes for breastfeeding-mediated immune benefits remains controversial. To identify the current State-of-the-Art on breastmilk leukocyte research, unearth knowledge gaps, and propose research priorities, a scoping review is necessary.

METHODS AND ANALYSIS

This scoping review will address the general question of what is known about leukocytes in human breastmilk. The development of this scoping review protocol adhered to the recommendations set forth by the Joanna Briggs Institute guidelines. Peer-reviewed research articles published in English, French, or Spanish will be eligible for inclusion in the scoping review. The initial literature search was conducted in January 2024 within the Medline, Embase, Cochrane central, and BVS databases.

ETHICS AND DISSEMINATION

This review does not require ethics approval. Our dissemination strategy includes peer review publication, presentations at conferences and to relevant stakeholders.

REGISTRATION DETAILS

This protocol was registered in Open Science Framework (available at: https://osf.io/kwfsy) on February 19th, 2024.

ARTICLE SUMMARY

Strengths and limitations of this study

- This scoping review represents the first systematic exploration of the literature on leukocytes in human breastmilk, underscoring the novelty of this research.
- We propose a novel system for quality appraisal of the literature on qualitative and quantitative characterization of breastmilk leukocytes.
- We will focus our analysis on peer-reviewed literature only, deposited in 4 databases, and published either in English, French or Spanish. Therefore, some relevant publications indexed in other places or in a different language may be missed.

Keywords: Colostrum, leukocytes, breastfeeding, breastmilk, immune cells, human milk

Introduction

Breastfeeding is encouraged by multiple agencies worldwide to promote health in both infants and mothers. Beyond aiding development through nutrition, breastmilk provides maternally-derived immune factors to suckling infants, including antibodies, cytokines, and growth factors [1–3]. These components protect infants from infectious diseases, sustain the maturation of the intestine, and promote the establishment of the commensal microbiota [4,5].

In addition to soluble factors, live immune cells, also called leukocytes, have been found in breastmilk. The composition of breastmilk leukocytes (BreLeuk) is dynamic and varies together with mother and infant health, maternal BMI, and gestational age at birth, among other conditions impacting the mother-infant dyad [5–7]. Along the process of lactation, from the development of the mammary glands until involution, leukocytes of multiple lineages including lymphocytes, granulocytes, monocytes, and macrophages travel from peripheral tissues to the maturating mammary gland by blood and lymph, reside *in situ,* and are found in breastmilk [8–10]. The exact process regulating the presence of leukocytes in breastmilk remains elusive.

A variety of functions or roles have been proposed for BreLeuk. For instance, B lymphocytes produce the antibodies responsible for the passive immunity provided by breastmilk. While antibodies have been shown to travel through the transcytosis route from mammary tissue into breastmilk, antibody-producing B lymphocytes have also been described in breastmilk [11,12]. In mice, BreLeuk could survive the digestion process and migrate to various tissues from suckling pups, effectively creating microchimerism [13,14]. Additional animal studies have described a specific T lymphocyte subpopulation with effects on the intestinal microbiota and the local immune system that may persist across multiple generations, potentially through factors transferred through breastmilk [15]. Unique lactation-induced macrophages were recently described in mouse breastmilk to also impact the microbiota establishment [16]. BreLeuk could directly participate in microbiota and pathogen regulation *in situ* through their multiple functions, for example phagocytosis [5].

Therefore, scattered information has documented BreLeuk in a variety of contexts, and BreLeuk may play relevant roles directly in the milk within the mammary gland or once ingested in the infant's intestine. Available reports are not comprehensive, focusing on a limited number of leukocyte lineages at a time, and/or maternal-infant conditions. Additionally, a limited number of studies pertain to the human species. The heterogeneity of breastmilk composition during lactation further complicates painting a comprehensive picture of the current landscape in human BreLeuk science [8]. Given the probable relevance of BreLeuk for promoting health in suckling infants, we propose here a protocol for a scoping review to systematically review the scientific literature reporting on human BreLeuk.

A search in PROSPERO, Open Science Framework, MEDLINE, Embase, Cochrane Database of Systematic Reviews and BVS was conducted, and no review on this topic was found the time of writing (January 2025). The objective of this scoping review is to

identify and organize the available information from the peer-reviewed original scientific literature about maternal leukocytes (including but not restricted to proportions, concentrations, subsets and described functions) in human colostrum/transitional and mature milk.

Methods and analysis

Study design

The aim of this protocol is to answer the research question formulated as:
"*What is known about human breastmilk leukocytes present in human milk during lactation?"*

The purpose of this review is to synthesize relevant qualitative and quantitative data from experimental research studies reporting on human BreLeuk. We will select peer reviewed research articles containing information on any human BreLeuk lineage and report the information together with its associated metadata. The proposed scoping review protocol has been developed following the JBI guidance for scoping reviews [17], and has been registered with Open Science Framework (https://osf.io/kwfsy). In addition, this protocol complies with the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) Statement for protocols, for all items that apply to scoping reviews (**Supplementary table 1**) [18]. We formulated the search strategy based on our research question, following the Population, Concept, and Context (PCC) framework as shown in **Table 1.**

Sources

The 4 selected databases for articles retrieval are Medline (OVID), Embase, Cochrane Central Register of Control Trials and BVS.

The relevant studies considered for this review will exclusively be peer-reviewed, published primary research articles. Study designs eligible encompass experimental and quasi-experimental, including randomized and non-randomized controlled trials, and analytical observational studies (prospective case-control studies and analytical cross-sectional studies). Case series, individual case reports and descriptive cross-sectional studies are also eligible. Excluded studies include opinions, reviews, gray literature, conference abstracts, and books. Articles included in the final output must satisfy the criteria described in **Table 2.** If a relevant article is not freely available online, support will be sought from the participants' institutional library services and by contacting the article's corresponding author(s).

Search strategy

On January 25th, 2024, an exploratory search was undertaken by an expert librarian (S.D.), restricted to the Medline database. The strategy involved applying keywords, and medical subject headings (MeSH) terms that associate to relevant key concepts, together with Boolean terms “AND” and “OR”. The MeSH terms used were the following: "Milk, Human", "Lactation", "Breast Feeding", "Colostrum", "Leukocytes", "Lymphocytes", "Killer Cells, Natural", "Monocytes", "Dendritic Cells", "Granulocytes", "Neutrophils",

"Eosinophils", "Basophils", "Natural Killer T-Cells", "B-Lymphocytes". The free terms used for this initial search were: Human milk, mature milk, breast milk, Maternal milk, transitional milk, Foremilk, Hindmilk, breastmilk, breastfeed*, "Breast feed*", Maternal transfer, Colostr*, Lactation, leukocyte*, lymphocyte*, B-Lymphocytes, myeloid, lymphoid, "Natural Killer", "NK Cells", Monocytes, Macrophage*, "dendritic cell*", "White blood cells", "White blood Corpuscle", "Immun* cells", Granulocytes, Neutrophil*, Eosinophil*, Basophil, "B cells", "T cells". The search was restricted to the human species; however, the exploratory search retrieved a very large proportion of non-human animal studies prompting iterations to the search strategy. Nine known relevant publications proposed by BreLeuk expert team members were confirmed to be present in the search output, which validated its scope.

The final search was performed from inception to January 31st, 2024, in the specified databases using the search string detailed in **Supplementary Appendix 2.** The strategy was then adapted by the expert librarian to the 3 additional databases (**Supplementary Appendices 3-5).**

Selection of sources of evidence

A total of 4193 articles were originally retrieved from the 4 databases and loaded to Rayyan platform [19], where 291 duplicates were deleted, giving a final selection of 3953 articles available for screening. The first screening process will be conducted blindly and independently by 3 researchers from the team (B.J., C.G., E.S.), using Rayyan. Screening will involve scrutiny of titles and abstracts of each article according to the selection criteria described in **Table 2**. Discrepancies in screening will be resolved by consensus after a thorough revision involving an additional topic expert from the team (M.B.). The second screening will be based on the full text version of each article to confirm relevance and will be performed by 3 team members (B.J., C.G., E.S.), independently. A preliminary flowchart of this process is described in **Figure 1**, that follows the Prisma-ScR guidelines [20].

Charting the data

Once the final list of articles to be included in this scoping review is complete, the full text versions of the articles will be scrutinized to collect relevant metadata. This will be done by 3 team members (B.J., C.G., and E.S.), independently, and the data will be charted automatically into an excel document using a Google form. Advantages of using a google form for the data charting process includes compulsory filling of information, leading to no empty fields that may cause confusion between unavailable data and the answer "No". It also allows the team to be working simultaneously, blindly and unambiguously on the same publication. The google form questionnaire elaborated to collect information for the scoping review is described in **Table 3.** While various questionnaire items may seem redundant (for example, Q24 and Q25), this will allow a more robust description of the studies (in the previous example, "colostrum" labelling has been used to describe breastmilk collected <2 days postpartum in some studies, but until <7 days postpartum in others [7,21–23]).

Analysis and presentation of results

We will adhere to the PRISMA-ScR guidelines to report the findings from this study [19]. The full list of included articles will be provided as part of the publication. The results of the scoping review will be categorized according to relevant themes including but not restricted to: study demographics, methodologies, and leukocyte subtype. These results will be presented as evidence maps, such as tables and diagrams, and narrated in text.

Metadata will be summarized to provide a comprehensive overview of the field, and specific relevant findings will be detailed, including frequency and concentration of leukocyte subtype in milk.

Sub-themes regarding the heterogeneity of populations (demographics), or maternal health status may emerge and will be described and discussed, as relevant.

Evidence quality appraisal

We anticipate the retrieved articles to be mostly observational studies. Given the unavailability of a quality assessment tool for the data in our scoping review, we developed a tool based on the JBI Critical Appraisal Checklist [24], merged with the adaptation of the Cochrane risk of bias tool, done by Wylde and colleagues in 2017 [25] . Our tool will grade each included paper for risk of bias, from 0 (no identified risk) to 1 (low risk, minor inconsistencies) to 2 (high risk, major inconsistencies), in answering the 5 questions below:

1. Were the criteria for mother inclusion in the study clearly defined?
2. Were technical confounding factors identified? (e.g. viability marker not used for flow cytometry, etc.)
3. Were quantitative data provided for all analyzed leukocytes declared in methods?
4. Were the methods described in sufficient details to allow reproducible experiment?
5. Other bias

Patient and Public Involvement

Patients and public were not involved in this work.

Discussion

Breastfeeding is a time-restricted opportunity to positively impact short- and long-term health. As research moves from empirical evidence to mechanistic explanations for the known benefits of breastmilk, BreLeuk emerges as enigmatic participants to breastmilk mediated health. The scoping review proposed in this protocol will help summarize what is known about leukocytes in human breastmilk, providing quantitative and qualitative evidence to help direct future research efforts.

Ethics and dissemination

This review does not require ethics approval. Our dissemination strategy includes peer review publication, presentations at conferences and to relevant stakeholders.

Footnotes

Availability of data and material

Additional data can be found in Supplementary materials.

Competing interest statement

The authors do not have any competing interest to declare.

Funding statement

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Author contributions

C.G. and B.J. produced the preliminary search strategy, wrote and edited the protocol and developed the data charting form. S.C.D., J.Y.N. and A.M.L. produced and edited the exploratory search and final search strategies, and produced and edited the manuscript. M.B. and E.S. conceived the study, wrote and edited the manuscript, and M.B additionally secured research funding. All authors approved the final version of the manuscript. MB is the guarantor of this work.

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Figures

Figure 1. PRISMA flow diagram

Tables

Table 1. Population-Concept-Context

Population	Any human study reporting data from leukocytes in human milk will be included, irrespective of maternal demographic or clinical status.
Concepts	Literature reporting on leukocytes (including, but not limited to granulocytes, monocytes, macrophages, B and T lymphocytes, dendritic cells, natural killers, and their progenitors), their measurements and identification in all stages of human milk (colostrum, transitional and mature milk) will be included in the scoping review. Associated metadata reporting on maternal/infant demographics and clinical characteristics, sample collection, milk and cell processing for analysis will also be charted.
Context	This review will examine any reported scenarios of human milk analyzed for the proportions, counts, or phenotype of any leukocyte subpopulation. The geographical location, stages of milk maturation (colostrum, transition and mature milk), maternal or infant health status, or study designs will not be limited.

Table 2. Inclusion and Exclusion Criteria

	Inclusion	Exclusion
Article type	Published peer-reviewed research articles	Review articles, pre-print articles, conference abstracts, grey literature
Language	English, Spanish & French	All languages other than English, Spanish & French
Country	All countries	None
Publication date	From inception until January 31st, 2024	Studies published after January 31st, 2024
Model of research	Human	Other than human
Cell population of characterization/evaluation	Leukocytes, including, but not restricted to: • Neutrophils • Eosinophils	Any other cell type present in breast milk including hematopoietic stem cells

	• Basophils • Monocytes • Macrophages • B lymphocytes • T lymphocytes • Dendritic cells • Natural killers	
Milk sampling	Any type of human milk (colostrum, transitional milk or mature milk), at any time of sampling (pre- and post-feeding, mornings/evenings, etc).	No restriction
Demographic & clinical data of the cohort participants	Human mothers, mothers of all ages, nationalities, health stages. All types of delivery Any neonate sex. Any neonatal term (pre-term, full term, post-term Any maternal or infant condition)	No restriction
Extras	Maternal pathologies or special medical conditions	No restriction

Table 3. Metadata: charting items

Publication information
Q1. Complete publication title
Q2. Authors
Q3. Nomenclature of the article from our database
Q4. Language
Q5. Keywords used
Study design *Please describe the parameters of the study in more detail (e.g. follow-up length, intervention, etc...)*
Q6. What was the overall aim of this study

Q7. Study type Observational study, intervention, cross-sectional, longitudinal, retrospective, prospective and/or other)
Q8. Complete cohort size (number of mothers included in the study)
Q9. Subgroups description (e.g. obese vs. lean) and size
Q10. Patients' dropout (only in clinical trials) --> If not relevant: NA, if relevant, add number of dropouts
Q11. Are infants also considered/described in this work?

Demographic & clinical data of the cohort participants

Q12. Nationality of the mothers
Q13. Age range of mothers (e.g. 18-38)
Q14. Health status of the test group of mothers (preeclampsia, gestational diabetes, COVID-19, HIV and/or other)
Q15. Additional information about mothers? (Ethnicity, socio-economic background, BMI, recruitment from a special group, etc...)
Q16. Recorded maternal medications or supplements of any kind (even outside of an intervention)
Q17. If infants were considered, what specific health status are included? (Healthy, term, pre-term, infections and/ or other)
Q18. If infants with infections were considered, which pathogen(s) is/are involved?
Q19. If infants with non-infectious diseases were included, describe which ones
Q20. Delivery method (vaginal delivery or C-section)
Q21. Gestational age (if available, in weeks)
Q22. Maternal food intake? No or yes with details.
Q23. Number of delivery of the mother

Milk sampling information

Q24. Milk type description (colostrum, transitional milk, mature and/or other)
Q25. Timing of sample collection postpartum (in number of days)
Q26. Information on exact timing of collection within the day (e.g. morning, pre-or post-infant feeding, 30min into feeding, etc...)
Q27. Methodology for collection (manual vs. automatic pump, cleaning of the area, discard first drops, etc...)
Q28. Range of volumes collected. Or exact volume if the case may be
Q29. Processing method (e.g. length of time between collection and processing, length of processing post collection (could affect neutrophil measurements, leukocyte activation, etc..), dilutions (in what buffer/medium?), centrifugation speed? discard supernatant? enrichment of populations (by density gradient, magnetic beads, etc..?), temperature of processing)
Q30. Long-term storage temperature
Q31. Other bioactives measured from milk samples?

Q32. Any confoundable aspects in the methodology details?

Leukocyte analysis*

We have 8 defined questions (Q34-Q41) aimed to gather as much information as possible of each analyzed leukocyte. Reactives Q41-Q121 correspond to the repetition of these 8 questions, available for as many leukocyte subtypes reported in each publication.

Q33. All immune cell populations of interest in this study

Q34. Immune cell population of interest in this section (First)

Q35. Description of the regulation of the phenotype of that cell in paper

(expression of surface molecules, cytokines, modification of function)

Leukocyte #1

Q36. Type of analysis performed (flow cytometry, microscopy, transcriptomic analysis and/or other)

Q37. In the case of flow cytometry analyses, indicate what gating strategy was used to identify each population

Q38. In the case of flow cytometry specify all that apply: (Use/not use of viability marker, gating/not gating on single cells, use/not use of FMOs, gating strategy provided/not provided as a figure, clear/unclear parental population description)

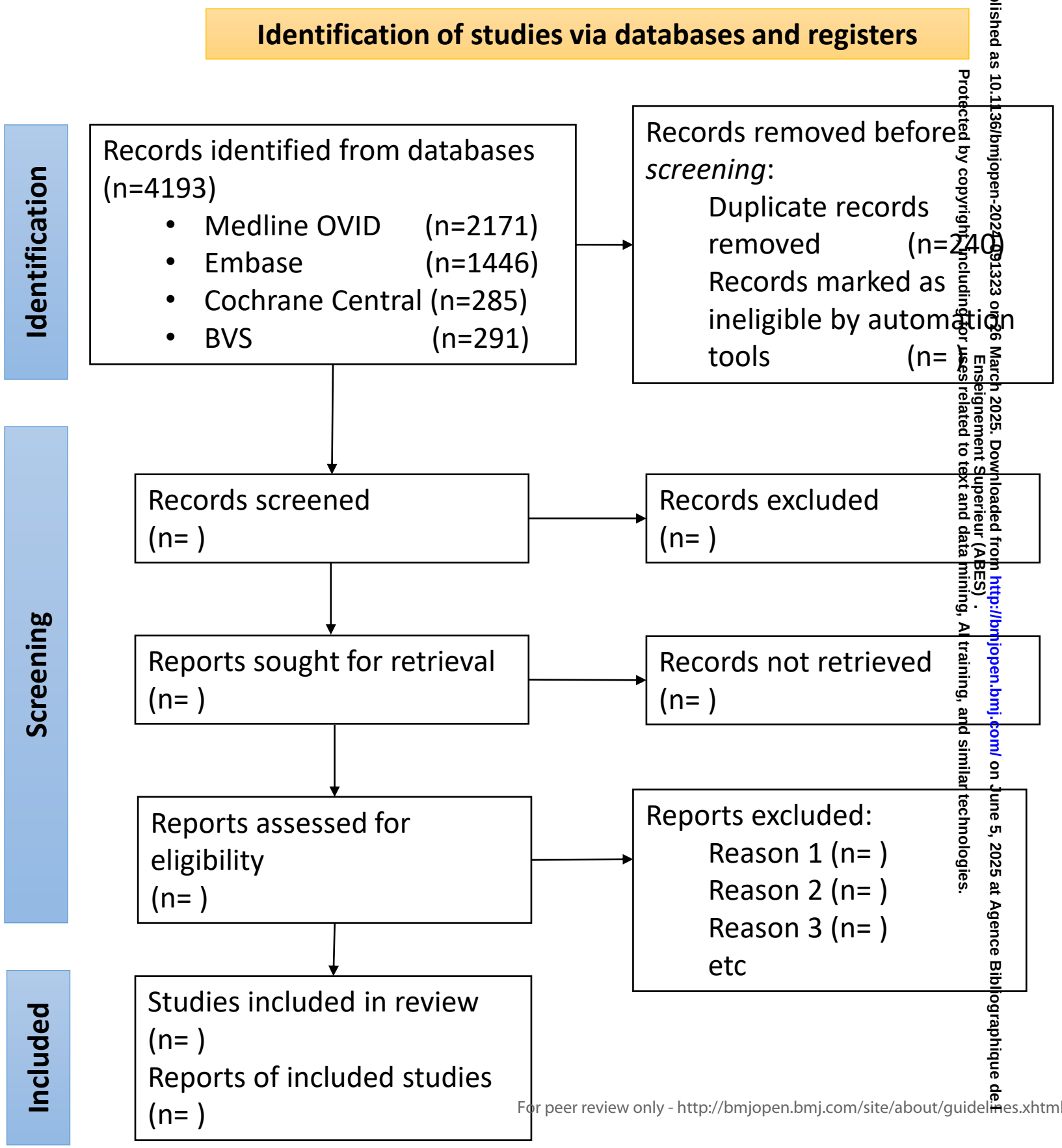
Q39. Additional methodology details

Q40. Methodology and results (Identity or phenotype modification (concentrations, phenotype, changes between groups/treatments, other relevant results on leukocytes in milk)

Q41. Additional information of interest outside of current categories

Q42-Q121. Repetition of the questions Q33 to Q41 for the different leukocytes that are described in the studies.

Figure 1



PRISMA-P 2015 Checklist for Scoping review protocol: Mapping the current knowledge on leukocytes in human breastmilk

This checklist has been adapted for use with protocol submissions to *Systematic Reviews* from Table 3 Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review **Our protocol is designed specifically to undertake a scoping review**	☒	☐	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	☒	NA
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	☒	☐	55-56
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	☒	☐	3-28
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	☒	☐	233-238
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	☐	☒	NA
Support					
Sources	5a	Indicate sources of financial or other support for the review	☒	☐	229-232
Sponsor	5b	Provide name for the review funder and/or sponsor	☐	☒	NA
Role of	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☐	☒	NA

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
sponsor/funder					
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	☒	☐	90-99
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO) **We used Population, Concepts, Context (PCC) instead**	☒	☐	108-110, 131-133
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review **Inclusion criteria was described according to PCC**	☒	☐	131-133
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	☒	☐	123-124
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	☒	☐	138-157
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	☒	☐	171-183
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	☒	☐	159-169
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	☒	☐	171-183
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☒	☐	Table 3
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	☐	☒	NA
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	☐	☒	NA

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☒	☐	186-189
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	☐	☒	NA
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☐	☒	NA
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	☒	☐	185-193
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☐	☒	NA
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☒	☐	195-203

Supplementary Appendix 2. Search strategy for Medline

Characteristic	Report
Type of search	New
Database	Medline
Platform	Ovid
Search date	03/02/2024
Date range for the search	No filter
Language restrictions	No filter
Other limitations	None
Search strategy	1. exp "Milk, Human"/ (23092) 2. exp "Lactation"/ (48800) 3. exp "Breast Feeding"/ (44731) 4. exp "Colostrum"/ (6930) 5. ((Human or mature or breast or Maternal or transitional) adj5 milk).tw. (28502) 6. Foremilk.tw. (244) 7. Hindmilk.tw. (85) 8. breastmilk.tw. (1721) 9. breastfeed*.tw. (29711) 10. "Breast feed*".tw. (13265) 11. (Maternal adj3 transfer).tw. (2152) 12. Colostr*.tw. (8455) 13. Lactation.tw. (37879) 14. or/1-13 (138862) 15. exp "Leukocytes"/ (830044) 16. exp "Lymphocytes"/ (579306) 17. exp "Killer Cells, Natural"/ (48402) 18. exp "Monocytes"/ (69247) 19. exp "Dendritic Cells"/ (54566) 20. exp "Granulocytes"/ (154790) 21. exp "Neutrophils"/ (97208) 22. exp "Eosinophils"/ (26104) 23. exp "Basophils"/ (8038) 24. exp "Natural Killer T-Cells"/ (3244) 25. exp "B-Lymphocytes"/ (104787) 26. leukocyte*.tw. (150835) 27. lymphocyte*.tw. (348191) 28. B-Lymphocytes.tw. (25520) 29. myeloid.tw. (109500) 30. lymphoid.tw. (85385) 31. ("Natural Killer" or "NK Cells").tw. (59121) 32. Monocytes.tw. (76287) 33. Macrophage*.tw. (289725) 34. "dendritic cell*".tw. (67588) 35. ("White blood" adj4 (cells or Corpuscle)).tw. (12908) 36. "Immun* cells".tw. (75475)

	37. Granulocytes.tw. (23427) 38. Neutrophil*.tw. (159175) 39. Eosinophil*.tw. (79470) 40. Basophil.tw. (5642) 41. "B cells".tw. (80857) 42. "T cells".tw. (254893) 43. or/15-42 (1526669) 44. 14 and 43 (4772) 45. 44 not ((animals.sh. or nonhuman.tw. or exp animals/) not humans.sh.) (2372) 46. (animals/ or Models, Animal/ or Disease Models, Animal/) not Humans/ (5155715) 47. ((animal or animals or canine* or cattle or bovine or buffalo or dog or dogs or feline or hamster* or lamb or lambs or mice or monkey* or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or cow* or veterinar*) not (human* or patient)).ti,kw,jw. (2464566) 48. 46 or 47 (5460647) 49. 45 not 48 (2171)
References	2171

Supplementary Appendix 3. Search strategy for Embase

Characteristic	Report
Type of search	New
Database	Embase
Platform	Embase.com
Search date	31/01/2024
Date range for the search	No filter
Language restrictions	No filter
Other limitations	None
Search strategy	1. 'breast milk'/exp (36631) 2. 'lactation'/exp (65746) 3. 'breast feeding'/exp (69736) 4. 'colostrum'/exp (10006) 5. ((human OR mature OR breast OR maternal OR transitional) NEAR/5 milk):ab,ti (38502) 6. foremilk:ab,ti (294) 7. hindmilk:ab,ti (127) 8. breastmilk:ab,ti (3888) 9. breastfeed*:ab,ti (52592) 10. 'breast feed*':ab,ti (17528) 11. (maternal NEAR/3 transfer):ab,ti (2894) 12. colostr*:ab,ti (10140) 13. lactation:ab,ti (49637) 14. #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 (189970)

15. 'leukocyte'/exp (1531736)
16. 'lymphocyte'/exp (1075920)
17. 'natural killer cell'/exp (102952)
18. 'monocyte'/exp (142898)
19. 'dendritic cell'/exp (130813)
20. 'granulocyte'/exp (273455)
21. 'neutrophil'/exp (179372)
22. 'eosinophil'/exp (63814)
23. 'basophil'/exp (17658)
24. 'natural killer t cell'/exp (13247)
25. 'b lymphocyte'/exp (224780)
26. leukocyte*:ab,ti (217318)
27. lymphocyte*:ab,ti (501699)
28. 'b lymphocytes':ab,ti (34133)
29. myeloid:ab,ti (199944)
30. lymphoid:ab,ti (124380)
31. 'natural killer':ab,ti OR 'nk cells':ab,ti (93279)
32. monocytes:ab,ti (117481)
33. macrophage*:ab,ti (416217)
34. 'dendritic cell*':ab,ti (106347)
35. ('white blood' NEAR/4 (cells OR corpuscle)):ab,ti (22717)
36. 'immun* cells':ab,ti (130081)
37. granulocytes:ab,ti (32973)
38. neutrophil:ab,ti (159042)
39. eosinophil*:ab,ti (130677)
40. basophil:ab,ti (9035)
41. 'b cells':ab,ti (126024)
42. 't cells':ab,ti (402796)
43. #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40 OR #41 OR #42 (2402009)
44. #14 AND #43 (7932)
45. #44 AND ('Conference Abstract'/it OR 'Conference Paper'/it OR 'Conference Review'/it) (1337)
46. (animal:ti,ab,lnk,kw,tn,tt,df,mn,dn OR animals:ti,ab,lnk,kw,tn,tt,df,mn,dn OR canine*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR cattle:ti,ab,lnk,kw,tn,tt,df,mn,dn OR bovine:ti,ab,lnk,kw,tn,tt,df,mn,dn OR buffalo:ti,ab,lnk,kw,tn,tt,df,mn,dn OR dog:ti,ab,lnk,kw,tn,tt,df,mn,dn OR dogs:ti,ab,lnk,kw,tn,tt,df,mn,dn OR feline:ti,ab,lnk,kw,tn,tt,df,mn,dn OR hamster*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR lamb:ti,ab,lnk,kw,tn,tt,df,mn,dn OR lambs:ti,ab,lnk,kw,tn,tt,df,mn,dn OR mice:ti,ab,lnk,kw,tn,tt,df,mn,dn OR monkey*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR mouse:ti,ab,lnk,kw,tn,tt,df,mn,dn OR murine:ti,ab,lnk,kw,tn,tt,df,mn,dn OR pig:ti,ab,lnk,kw,tn,tt,df,mn,dn OR pigs:ti,ab,lnk,kw,tn,tt,df,mn,dn OR piglet*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR porcine:ti,ab,lnk,kw,tn,tt,df,mn,dn OR primate*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR rabbit*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR rats:ti,ab,lnk,kw,tn,tt,df,mn,dn OR rat:ti,ab,lnk,kw,tn,tt,df,mn,dn OR rodent*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR sheep*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR cow*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR veterinar*:ti,ab,lnk,kw,tn,tt,df,mn,dn) NOT

	(human*:ti,ab,lnk,kw,tn,tt,df,mn,dn OR patient:ti,ab,lnk,kw,tn,tt,df,mn,dn) (4569866) 47. #45 NOT #46 (973) 48. #44 NOT #45 (6595) 49. ('animal'/exp OR 'juvenile animal'/exp OR 'adult animal'/exp OR 'animal cell'/exp OR 'animal tissue'/exp OR 'nonhuman'/exp OR 'animal experiment'/exp OR 'animal model'/exp) NOT 'human'/exp (8024124) 50. #48 NOT (#46 OR #49) (3295) 51. #47 OR #50 (4268) 52. #51 AND [embase]/lim NOT ([embase]/lim AND [medline]/lim) (1446)
References	1446

Supplementary Appendix 4. Search strategy for Cochrane Central Register of Controlled Trials

Characteristic	Report
Type of search	New
Database	Cochrane Central Register of Controlled Trials
Platform	Ovid
Search date	31/01/2024
Date range for the search	No filter
Language restrictions	No filter
Other limitations	None
Search strategy	1. exp "Milk, Human"/ (1274) 2. exp "Lactation"/ (938) 3. exp "Breast Feeding"/ (2639) 4. exp "Colostrum"/ (216) 5. ((Human or mature or breast or Maternal or transitional) adj5 milk).tw. (4026) 6. Foremilk.tw. (8) 7. Hindmilk.tw. (16) 8. breastmilk.tw. (395) 9. breastfeed*.tw. (6571) 10. "Breast feed*".tw. (2833) 11. (Maternal adj3 transfer).tw. (69) 12. Colostr*.tw. (556) 13. Lactation.tw. (2862) 14. or/1-13 (14866) 15. exp "Leukocytes"/ (11224) 16. exp "Lymphocytes"/ (6319) 17. exp "Killer Cells, Natural"/ (878) 18. exp "Monocytes"/ (850) 19. exp "Dendritic Cells"/ (368) 20. exp "Granulocytes"/ (2844) 21. exp "Neutrophils"/ (1587) 22. exp "Eosinophils"/ (929) 23. exp "Basophils"/ (155)

	24. exp "Natural Killer T-Cells"/ (17) 25. exp "B-Lymphocytes"/ (634) 26. leukocyte*.tw. (6984) 27. lymphocyte*.tw. (13034) 28. B-Lymphocytes.tw. (431) 29. myeloid.tw. (6405) 30. lymphoid.tw. (1172) 31. ("Natural Killer" or "NK Cells").tw. (2694) 32. Monocytes.tw. (2321) 33. Macrophage*.tw. (4471) 34. "dendritic cell*".tw. (1618) 35. ("White blood" adj4 (cells or Corpuscle)).tw. (1613) 36. "Immun* cells".tw. (1907) 37. Granulocytes.tw. (725) 38. Neutrophil*.tw. (12778) 39. Eosinophil*.tw. (5730) 40. Basophil.tw. (417) 41. "B cells".tw. (1883) 42. "T cells".tw. (6638) 43. or/15-42 (55958) 44. 14 and 43 (308) 45. 44 not ((animals.sh. or nonhuman.tw. or exp animals/) not humans.sh.) (300) 46. (animals/ or Models, Animal/ or Disease Models, Animal/) not Humans/ (2656) 47. ((animal or animals or canine* or cattle or bovine or buffalo or dog or dogs or feline or hamster* or lamb or lambs or mice or monkey* or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or cow* or veterinar*) not (human* or patient)).ti,kw,jw. (6393) 48. 46 or 47 (7313) 49. 45 not 48 (285)
References	285

Supplementary Appendix 5. Search strategy for BVS

Characteristic	Report
Type of search	New
Database	LILACS, WPRIM, LIS, IBECS, BDENF. CUMED, LIPECS, SES-SP, BBO, BRISA, VETINDEX, colecionaSUS and INDEXPSI
Platform	BVS
Search date	31/01/2024
Date range for the search	No filter
Language restrictions	No filter
Other limitations	None
Search strategy	(((lactation OR "breast milk" OR breastmilk OR "breast feed*" OR "leche materna" OR colostr* OR calostro OR lactancia OR amamanta*)) AND ((leucocitos OR leukocytes OR linfocitos OR lymphocytes OR "células

	asesinas naturales" OR "natural killer cells" OR monocitos OR monocytes OR "células dendríticas" OR "dendritic cells" OR granulocitos OR granulocytes OR neutrófilos OR neutrophils OR eosinófilos OR eosinophils OR basófilos OR basophils OR "células T asesinas naturales" OR "natural killer T cells" OR "linfocitos B" OR "B lymphocytes" OR mieloide OR myeloid OR linfoide OR lymphoid OR macrófagos OR macrophages OR "white blood" OR "células B" OR "B cells" OR "células T" OR "T cells"))) AND NOT (ti:((animal OR animals OR canine* OR cattle OR bovine OR buffalo OR dog OR dogs OR feline OR hamster* OR lamb OR lambs OR mice OR monkey* OR mouse OR murine OR pig OR pigs OR piglet* OR porcine OR primate* OR rabbit* OR rats OR rat OR rodent* OR sheep* OR cow* OR veterinar*))) AND (db:("LILACS" OR "WPRIM" OR "LIS" OR "IBECS" OR "BDENF" OR "CUMED" OR "LIPECS" OR "SES-SP" OR "BBO" OR "BRISA" OR "VETINDEX" OR "colecionaSUS" OR "INDEXPSI"))
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