Article

Not peer-reviewed version

Weighted Epigenetic Profiling of Milk miRNAs Across Human, Bovine, and Camel Species: A Comparative Global Score

Maksym Zoziuk * , Abel Dafogo Djibagaou , Koutaya Dezoumbe , Noubaramadji Yamti Suitombaye ,
Amine Akouya , Abdirahman Ali , Giulia Cappelli , Dimitri Koroliouk , Maurizio Mattei , Maria Cristina Caroleo ,
Elena Ciani , Mohamed Ahmed Jimale , Vittorio Colizzi

Posted Date: 26 March 2026

doi: 10.20944/preprints202603.2048.v1

Keywords: milk microRNA; camel milk; exosomes; epigenetic programming; functional enrichment; STAT3 doi:10.20944/preprints202603.2048.v1

Article

Weighted Epigenetic Profiling of Milk miRNAs Across Human, Bovine, and Camel Species: A Comparative Global Score

Maksym Zoziuk [1,2,*], Abel Dafogo Djibagaou [3], Koutaya Dezoumbe [3], Noubaramadji Yamti Suitombaye [3], Amine Akouya [3], Abdirahman Ali [4], Giulia Cappelli [5], Dimitri Koroliouk [1,2], Maurizio Mattei [2], Maria Cristina Caroleo [6], Elena Ciani [7], Mohamed Ahmed Jimale [8] and Vittorio Colizzi [2,3]

[1] Kyiv Polytechnic Institute, Kyiv, Ukraine
[2] Tor Vergata University and Foundation, Rome, Italy
[3] Laboratoire des Grandes Epidemies Tropicales, Faculty of Medicine, University Hospital Complex "Bon Samaritain", N'Djamena, Chad
[4] Centre for Camel Research and Development, Faculty of Veterinary Medicine and Animal Husbandry, Somali National University KM7 Campus, Mogadishu, Somalia
[5] Institute of System Biology, National Research Council, Montelibretti-Rome, Italy
[6] Department of Health Sciences, University Magna Grecia, Catanzaro, Italy
[7] Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Italy
[8] Department of Animal Physiology, Faculty of Veterinary Medicine and Animal Husbandry, Somali National University KM7 Campus, Mogadishu, Somalia
* Correspondence: zzkmsy01@uniroma2.it

Simple Summary

Milk is increasingly recognized as a complex biological fluid containing regulatory molecules, such as microRNAs (miRNAs), that may influence gene expression in the consumer. While the roles of miRNAs in human and bovine milk are well-studied, the functional potential of camel milk miRNAs remains poorly characterized despite its reported therapeutic benefits. In this study, we performed a comparative systems-level analysis of miRNAs in human, bovine, and camel milk. We developed a weighted scoring framework—validated by a consensus of multiple predictive algorithms—to estimate their regulatory influence by integrating miRNA abundance with target binding affinity. Our results reveal a large conserved regulatory core shared across all species, primarily associated with growth signaling and neuronal development. Notably, we found that bovine milk is functionally more similar to human milk than camel milk is. In contrast, camel milk exhibits a highly specialized regulatory signature targeting key metabolic and anti-inflammatory pathways (such as STAT3 signaling). These findings suggest that different mammalian milks carry species-specific regulatory signals, providing a molecular basis for the unique health properties of camel milk and a new framework for nutritional epigenetics.

Abstract

Milk microRNAs (miRNAs) are key mediators of maternal–offspring communication, potentially influencing postnatal development through systemic epigenetic regulation. While miRNAs in human and bovine milk are extensively studied, the regulatory landscape of camel milk remains underexplored despite its unique therapeutic reputation. In this study, we conducted a comparative cross-species analysis of miRNA targets from human (*Homo sapiens*), bovine (*Bos taurus*), and camel (*Camelus dromedarius*) milk. To estimate regulatory impact, we implemented the Global Score, a weighted metric integrating quantitative miRNA abundance with thermodynamic binding affinity (miRWalk 2.0), cross-validated by multi-algorithm statistical consensus via mirDIP 5.2. Analysis revealed distinct species-specific miRNA concentration strategies: human milk maintains a highly diversified "regulatory symphony" (90% mass distributed across 35 miRNAs), while camel milk utilizes a "biological intervention" strategy, with only 12 miRNAs constituting 90% of the pool and miR-148a exerting extreme dominance (61.8%). A conserved regulatory core (Common All) was identified, accounting for over 80% of the total regulatory weight, primarily associated with neuroplasticity and MAPK/Ras signaling. Camel milk miRNAs displayed a distinctive therapeutic signature, uniquely targeting pivotal immune and metabolic nodes, specifically STAT3 (linked to Th17 cell differentiation) and PPARD, providing a molecular basis for its reported anti-inflammatory and anti-diabetic effects. Conversely, human milk showed high-confidence human-specific targeting of NLGN3, essential for synaptic organization. Bovine milk exhibited a significantly closer functional alignment to the human "epigenetic template" (14.8% shared genes) than camel milk (1.5%), particularly in pathways maintaining genomic stability (e.g., XPO1). These findings demonstrate that milk miRNAs operate through both a universal developmental backbone and specialized regulatory environments, offering a comparative framework for applications in clinical nutrition and precision medicine.

Keywords: milk microRNA; camel milk; exosomes; epigenetic programming; functional enrichment; STAT3

1. Introduction

Mammalian milk has evolved far beyond its traditional role as a source of macronutrients and micronutrients, functioning instead as a complex biological fluid that mediates biochemical communication between mothers and offspring. In addition to lipids, proteins, and carbohydrates, milk contains a diverse repertoire of bioactive molecules—including hormones, immune factors, extracellular vesicles, and regulatory RNAs—that contribute to postnatal physiological adaptation and long-term developmental programming. Among these components, microRNAs (miRNAs) have emerged as important regulators capable of modulating gene expression networks involved in metabolism, immune maturation, and cellular differentiation [1].

MiRNAs are short (~22 nucleotide) non-coding RNA molecules that regulate gene expression primarily through post-transcriptional repression of target messenger RNAs. In milk, miRNAs are predominantly packaged within extracellular vesicles, particularly exosomes, which confer protection against environmental degradation. This encapsulation allows miRNAs to withstand harsh gastrointestinal conditions, including low pH and enzymatic digestion [2]. Experimental evidence suggests that a fraction of these vesicle-associated miRNAs can be absorbed through the intestinal epithelium and enter systemic circulation in a biologically active form, where they may accumulate in peripheral tissues and influence host gene expression [3]. Although the extent and physiological relevance of dietary miRNA uptake remain debated, accumulating evidence supports the possibility that milk-derived miRNAs participate in interspecies molecular signaling.

Most current knowledge regarding milk miRNAs derives from studies on human and bovine milk, which have revealed conserved regulatory profiles associated with immune development, metabolic regulation, and epithelial barrier maturation. In contrast, the miRNA landscape of camel milk remains comparatively underexplored despite growing interest in its therapeutic potential. Camel milk is increasingly recognized in the scientific community as a ‘bioactive powerhouse’ due to its unique nutritional profile, more importantly, its distinct absence of *β-lactoglobulin*, the primary allergen in bovine milk, making camel milk a viable and safe dairy alternative for individuals with milk protein allergies. Therapeutically, camel milk has been widely reported to exhibit anti-inflammatory, immunomodulatory, and anti-diabetic effects, and is increasingly investigated as a functional food in metabolic and autoimmune disorders. However, the molecular mechanisms underlying these properties remain only partially characterized, and the possible contribution of miRNA-mediated regulation has received limited systematic investigation.

Another important limitation in the current literature concerns the analytical framework used to interpret milk miRNA function. Many studies emphasize the presence or relative abundance of specific miRNAs, but do not integrate quantitative expression levels with the strength of miRNA–mRNA interactions. Because the regulatory capacity of miRNAs depends not only on their presence but also on their concentration and binding affinity to target transcripts, analyses based solely on qualitative or relative abundance may overestimate the biological significance of certain miRNAs while overlooking highly abundant regulators with moderate affinity. Although some quantitative approaches have been proposed [4–7], comprehensive frameworks integrating expression levels with thermodynamic binding parameters remain scarce.

To address these limitations, we developed a robust weighted metric score, termed the 'Global Score', and performed a comparative cross-species analysis of milk miRNAs from human, bovine, and camel sources using an integrative scoring framework that combines quantitative miRNA abundance with predicted thermodynamic binding affinity to target genes.

This metric Global Score, provides an expression-weighted estimate of the regulatory potential of individual miRNA–target interactions. Functional interpretation was then performed using gene set enrichment analyses across Gene Ontology (GO), KEGG pathways, and MSigDB Hallmark gene sets to identify conserved and species-specific regulatory signatures.

Through this weighted system-level approach, we sought to identify both evolutionarily conserved regulatory signatures shared across mammalian milk and species-specific regulatory clusters that may explain functional differences among milk types. We hypothesized that camel milk miRNAs may display unique regulatory patterns associated with metabolic and inflammatory pathways, potentially contributing to the distinct biomedical properties attributed to this milk in clinical and nutritional contexts. By integrating quantitative expression data with thermodynamic interaction modeling and pathway-level interpretation, this study aims to provide a more rigorous framework for evaluating the regulatory potential of dietary miRNAs and to clarify the comparative functional landscape of human, bovine, and camel milk.

2. Materials and Methods

Data Acquisition and Quality Control

miRNA expression profiles from mammalian milk were obtained from publicly available high-throughput sequencing datasets. Camel milk miRNA sequences were retrieved from the dataset reported in [8], while human and bovine milk miRNA profiles were obtained from the dataset described in [9]. These studies used next-generation sequencing platforms for small RNA profiling and included biological replicates for each milk type.

Raw reading counts were extracted from the published datasets and averaged across biological replicates to obtain representative expression values for each miRNA within a given species. To enable cross-species comparison while minimizing the influence of low-abundance transcripts potentially arising from sequencing artifacts or stochastic transcriptional noise, miRNAs were ranked according to their read counts and cumulatively selected until reaching approximately 90% of the total read mass for each species.

This approach prioritizes highly expressed miRNAs that are more likely to exert biologically meaningful regulatory effects while excluding low-copy miRNAs with uncertain functional relevance. Based on this criterion, 36 miRNAs were retained for human milk, 19 for bovine milk, and 12 for camel milk.

For cross-species comparability, miRNA expression levels were normalized within each species by converting read counts into relative abundance percentages. Because the datasets originated from independent studies, this relative normalization approach was used to minimize potential batch effects and enable cross-species comparison.

Predicted miRNA target genes were identified using miRWalk 2.0 [10]. Only interactions with a binding score greater than 0.95 were considered. These results were cross-validated using the mirDIP

5.2 database [11]. We restricted the analysis to target genes classified within the "High" and "Very High" confidence classes (representing the top 5% of integrated predictions). This dual-database consensus approach—combining miRWalk's thermodynamic stringency with mirDIP's multi-tool statistical validation—ensures that the identified genes are not only thermodynamically plausible but also supported by an extensive algorithmic consensus, significantly reducing the likelihood of false-positive results.

Weighted Global Score approach

Because the regulatory impact of a miRNA depends on both its abundance and its predicted interaction score with target transcripts, we developed a composite metric termed the Global Score (1) to estimate the cumulative regulatory influence exerted by the miRNA pool on individual genes.

The Global Score was defined as:

$$W_g = \sum_{k=1}^{n}(E_i * S_i) \qquad (1)$$

where: Ei represents the relative expression level of the *i*-th miRNA (percentage of total reads), Si represents the miRWalk binding probability score for the interaction between miRNA *i* and the target gene.

The summation is performed across all miRNAs predicted to target the same gene. This formulation integrates miRNA expression levels with predicted interaction scores to estimate the overall regulatory influence exerted on each target gene. Because computational miRNA target prediction may generate false-positive interactions, the additional use of expression-weighted scoring was intended to further reduce spurious predictions and filter low-copy regulatory signals unlikely to exert biologically meaningful effects [12].

Classification of Targeted Genes

Target genes were classified into seven regulatory categories according to their cross-species regulatory profiles derived from the Global Score. Genes receiving regulatory contributions (Wg > 0) from all three milk miRNA pools were assigned to the Common All category. Genes targeted by two species only were grouped into three pairwise categories (Human_Cow, Human_Camel, and Cow_Camel), while genes receiving regulatory contributions exclusively from one species were assigned to the species-specific groups (Unique Human, Unique Cow, and Unique Camel).

Functional enrichment analysis was performed using the Enrichr platform across three annotation libraries: Gene Ontology Biological Process 2023, KEGG Pathways 2021, and MSigDB Hallmark 2020.

To incorporate the regulatory intensity derived from the miRNA–target interaction model, pathway enrichment results were further weighted using a Weighted Enrichment Score (We) that integrates Enrichr's Combined Score with the mean regulatory weight (mean Wg) of genes contributing to each pathway (2).

$$W_s = CS * \sum_{j=1}^{k}\frac{W_{g,j}}{k} \qquad (2)$$

where CS is the combined score from Enrichr (c = ln(p) * z, where p is the Fisher's exact test p-value and z is the z-score deviation). k is the number of target genes overlapping with the specific pathway (overlap). Wg,j is the regulatory weight of the j-th gene within that pathway. The term Σ(Wg,j/k) represents the mean regulatory weight of the genes involved in the pathway. This approach allows enrichment results to reflect not only the statistical overrepresentation of genes but also the predicted regulatory strength exerted by milk miRNAs on the corresponding biological processes.

doi:10.20944/preprints202603.2048.v1

3. Results

Analysis revealed distinct miRNA concentration strategies across species. Human milk showed the highest diversification, with 90% of the regulatory mass distributed among 35 miRNA species, where the leading miR-148a accounted for only 19.2% of the pool. In contrast, bovine and camel milk showed significantly narrower repertoires, reaching the 90% mass threshold with only 19 (leader: 41.7%) and 12 miRNAs, respectively. In camel milk, miR-148a exhibited extreme dominance, representing 61.8% of the entire pool. This shift indicates a transition from a multi-target developmental strategy in humans to a highly specialized "targeted" model in camels.

The distribution of target genes reveals a highly conserved regulatory architecture across mammalian exosomal miRNAs. The Common All core dominates the landscape, accounting for the vast majority of total regulatory weight – Figure 1. Beyond this core, a significant functional alignment is observed between bovine and human milk, with 14.8% of the remaining genes shared exclusively between these two species. By contrast, the Human–Camel overlap is notably smaller (1.5%), a finding with important implications for infant formula research and the selection of bovine milk as a primary human milk analog.

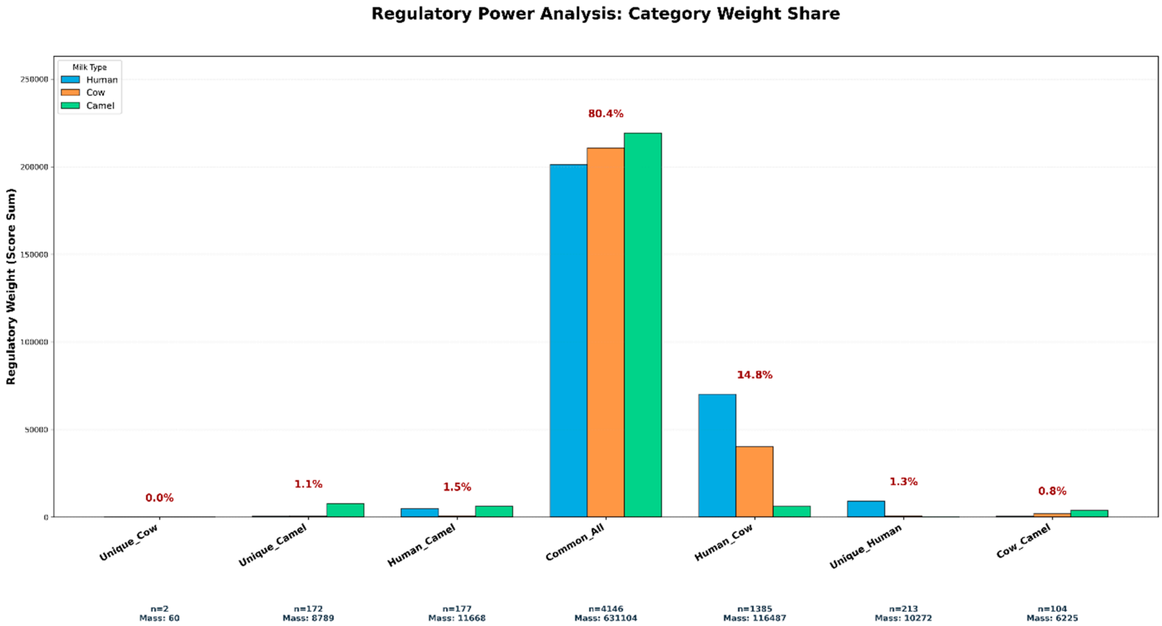


Figure 1. Classification of target genes by milk source and regulatory impact. The values for n indicate gene count per category; mass reflects the total weighted targeting score assigned to the respective gene sets.

Integrative cross-referencing with the mirDIP reinforces this hierarchy. High-weight consensus targets such as LCOR (Global Score: 197.5), USP47 (195.8), and CPEB4 (192.8) exhibit remarkably stable regulatory weights across all three species, confirming that the computational predictions align with a broad algorithmic consensus (Top 1-5% confidence classes). Important genes presented in Table 1. The evolutionarily conserved core (Common All - Weighted Enrichment Score > 25,000) establishes a universal regulatory backbone that dominates RNA polymerase II transcription and MAPK/Ras signaling, coordinating cell survival and growth. miRWalk identified RIMS2 (Global Score: 2160.85) and ELAVL4 as central drivers of synaptic transmission, alongside epigenetic regulators MBD1 (1710.75) and CUX1. Independent validation through mirDIP confirms the robustness of this core, particularly for signaling nodes like SMAD2 (179.3) and AGO1 (188.8). The high consensus for TSC1 (mTOR pathway) and MAPK10 further reinforces the existence of a stable regulatory environment for cellular differentiation that remains consistent regardless of the milk source, suggesting an evolutionary "tuning" of milk miRNAs toward fundamental developmental processes.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 March 2026 doi:10.20944/preprints202603.2048.v1

Table 1. Key Regulatory Drivers and Integrated Scores across Species Categories.

Category	Gene Symbol	miRWalk Global Score	mirDIP Global Score	Biological Function
Common All (Evolutionary Core)	SMAD2	1614.51	179.33	TGF-beta signaling; cell growth & differentiation
	LCOR	1064.05	197.52	Universal transcriptional corepressor
	AGO1	801.16	188.87	Essential component of RNAi machinery
Human_Cow (Shared Barriers)	XPO1	32.98	31.56	Nuclear-cytoplasmic transport (exportin 1)
	IKZF3	3619.62	—	B-lymphocyte differentiation & immunity
	VEGFA	755.69	11.20	Angiogenesis & tissue regeneration
Unique Camel (Immunometabolic)	STAT3	710.21	44.12	JAK-STAT / Th17 (anti-inflammatory signaling)
	PPARD	204.87	75.37	Insulin sensitivity & fatty acid metabolism
Unique Human (Specialization)	FAM111A	765.44	2.57	Systemic growth & cell differentiation
	NLGN3	227.15	2.67	Human-specific synaptic organization & adhesion
Cow_Camel	PRKAA1	155.78	114.56	AMPK energy sensor (Cow-Camel metabolic support)
Human_Camel	PSEN1	867.59	92.61	Protein processing (Human-Camel cognitive support)

All the data presented in Supplementary Materials.

Human_Cow category highlights a shared emphasis on adaptive immunity and DNA integrity between human and bovine milk. The leading drivers include IKZF3 (825.47), which coordinates B-lymphocyte differentiation, and PMS2 (720.3), which supports genetic stability through mismatch repair. The mirDIP results identify XPO1 (Nuclear transport) and BCL2L1 (Apoptosis inhibition) as highly validated shared targets. The alignment of these high-confidence nodes supports the hypothesis that bovine milk retains partial functional similarity to human milk, specifically in the domains of tissue regeneration (via VEGFA and FGFR2) and immune maturation.

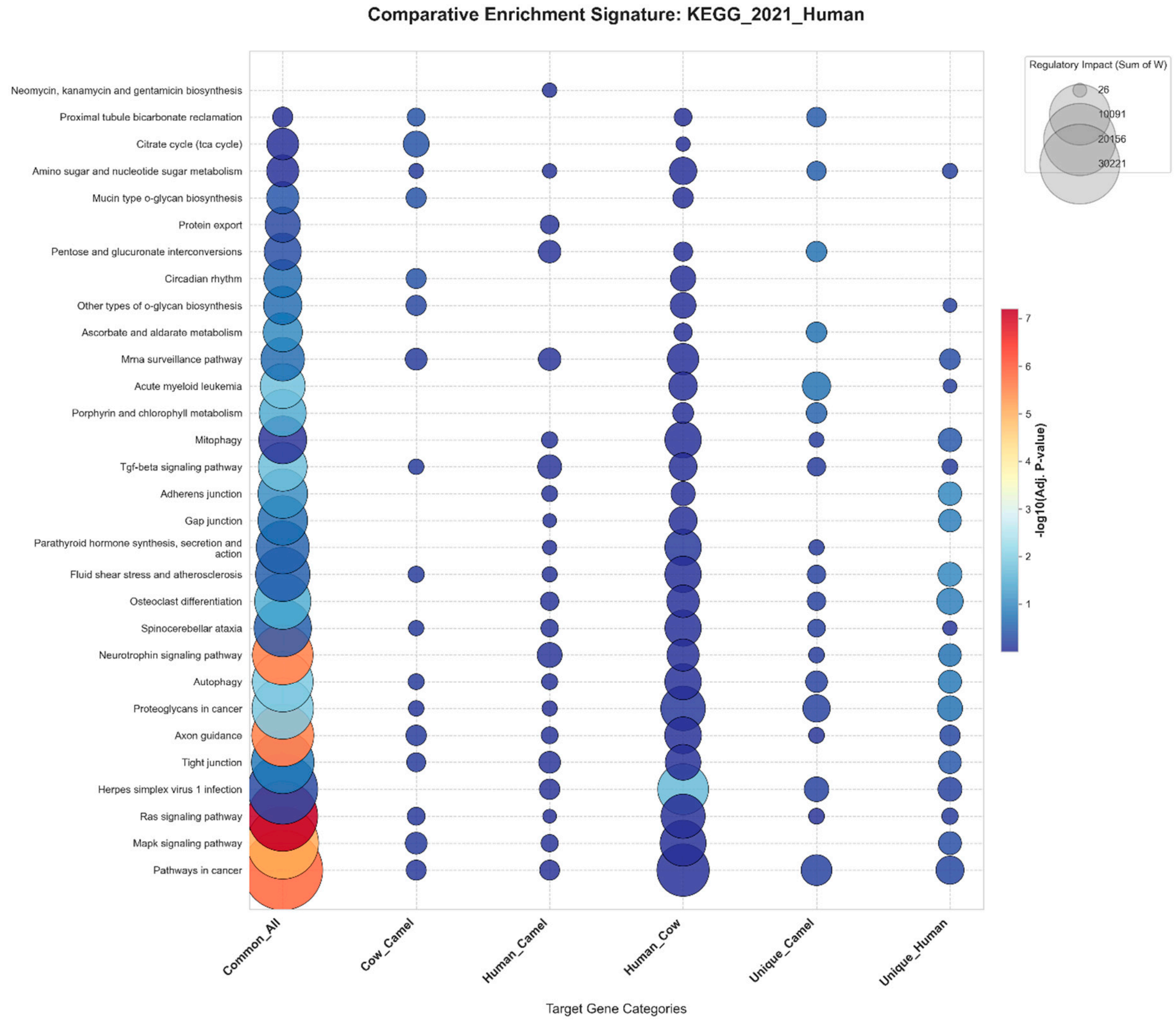
Camel milk exhibits an intense and specific regulatory profile, targeting genes linked to its reported clinical benefits. Key nodes include LARGE1 (314.6) and the critical therapeutic regulator STAT3 (287.2). Notably, STAT3 is corroborated by mirDIP as a "High Confidence" target. This dual-database confirmation provides a robust molecular basis for camel milk's unique anti-inflammatory properties, specifically through the modulation of Th17 cell differentiation and the JAK–STAT pathway. Additional metabolic potential is realized via FDFT1 (cholesterol biosynthesis) and PPARD (insulin sensitivity). The consistency between expression-based weights and statistical consensus for STAT3 and PPARD suggests that miRNA-mediated regulation is a primary mechanism underlying the distinct therapeutic effects of camel milk.

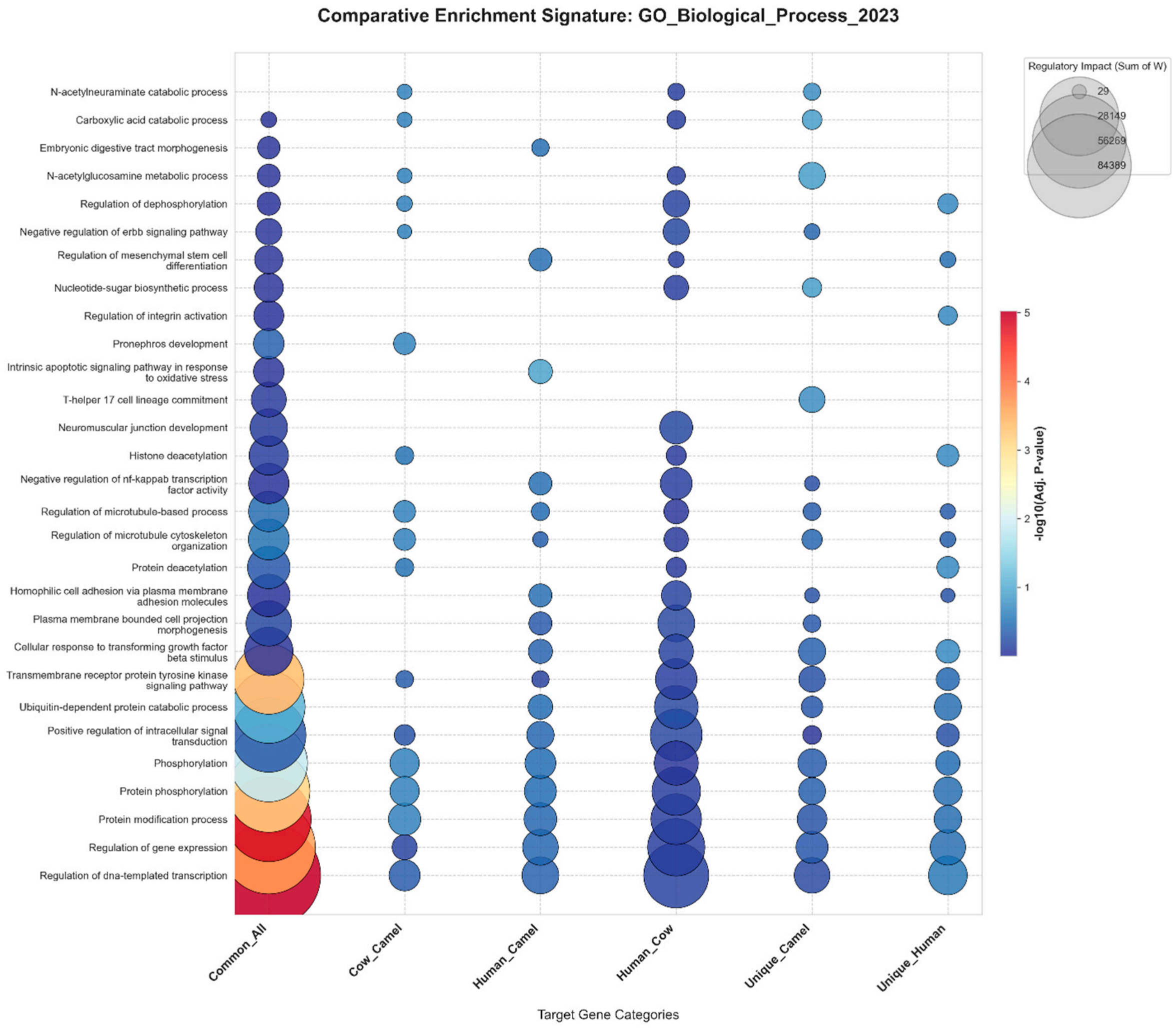
Human milk miRNAs uniquely focus on advanced neurodevelopment and epithelial barrier construction. miRWalk weighting emphasizes FAM111A (220.3) and MITF (172.7) as drivers of systemic growth. A point of perfect alignment between platforms is found in NLGN3 (Synaptic organization), which mirDIP identifies as a high-confidence target specifically within the human miRNA pool. Enrichment analysis highlights TGF-beta signaling and apical junction formation

(Score: 371.6), underscoring a specialized focus on tissue barrier integrity—consistent with the unique developmental demands of the human neonatal period.

The Cow_Camel cluster focuses on cellular energy balance, specifically the TCA cycle via DLAT and PRKAA1/AMPK signaling. The Human_Camel category centers on cognitive support through PSEN1 and SGIP1, aligning camel milk's functional profile with specific human neurodevelopmental needs.

While the universal core ensures basic biological consistency, species-specific miRNA profiles—validated by the intersection of thermodynamic probability and multi-algorithm consensus—create specialized regulatory environments tailored to unique physiological requirements – Figure 2.




Comparative Enrichment Signature: GO_Biological_Process_2023
N-acetylneuraminate catabolic process
Carboxylic acid catabolic process
Embryonic digestive tract morphogenesis
N-acetylglucosamine metabolic process
Regulation of dephosphorylation
Negative regulation of erbb signaling pathway
Regulation of mesenchymal stem cell differentiation
Nucleotide-sugar biosynthetic process
Regulation of integrin activation
Pronephros development
Intrinsic apoptotic signaling pathway in response to oxidative stress
T-helper 17 cell lineage commitment
Neuromuscular junction development
Histone deacetylation
Negative regulation of nf-kappab transcription factor activity
Regulation of microtubule-based process
Regulation of microtubule cytoskeleton organization
Protein deacetylation
Homophilic cell adhesion via plasma membrane adhesion molecules
Plasma membrane bounded cell projection morphogenesis
Cellular response to transforming growth factor beta stimulus
Transmembrane receptor protein tyrosine kinase signaling pathway
Ubiquitin-dependent protein catabolic process
Positive regulation of intracellular signal transduction
Phosphorylation
Protein phosphorylation
Protein modification process
Regulation of gene expression
Regulation of dna-templated transcription
Common_All
Cow_Camel
Human_Camel
Human_Cow
Unique_Camel
Unique_Human
Target Gene Categories
Regulatory Impact (Sum of W)
29
28149
56269
84389
-log10(Adj. P-value)
5
4
3
2
1

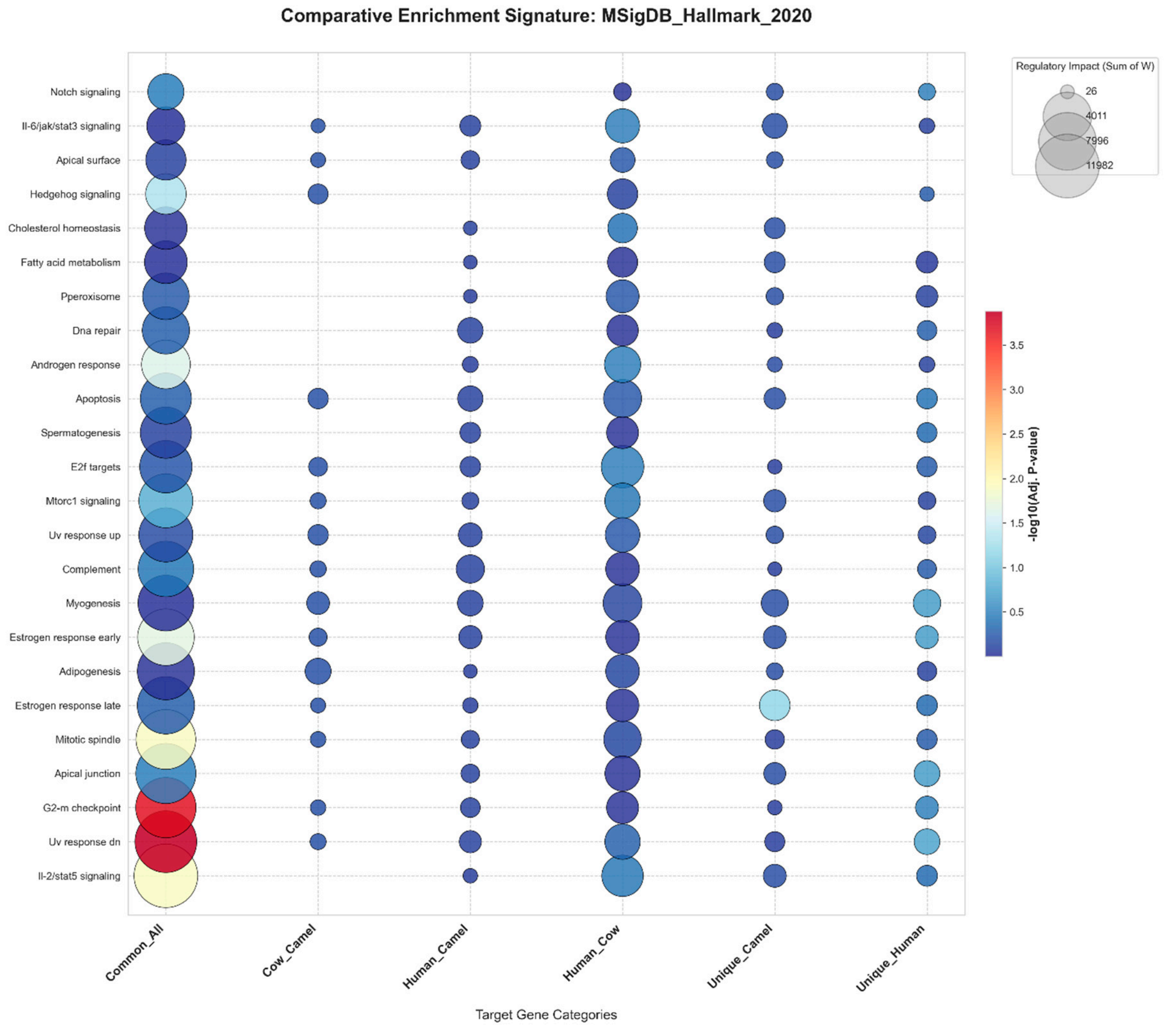


Figure 2. Integrated comparative analysis of GO, KEGG, and Hallmark signatures.

4. Discussion

The absorption of miRNAs by the human body primarily depends on their encapsulation within milk exosomes, which protect these molecules from degradation under low gastric pH and digestive enzymes [13]. Translocation through the intestinal epithelium is an active process mediated by receptor-dependent endocytosis or macropinocytosis, enabling vesicles to enter systemic circulation in a biologically active state [14]. While intestinal barrier permeability is highest in infants, systemic miRNA transport has been shown to persist into adulthood [15]. However, the degree of technological processing remains a critical factor, as pasteurization significantly reduces exosome stability and bioavailability compared to the native product [5].

Modern toxicological and nutritional approaches emphasize that the physiological impact of these molecules is strictly dose-dependent [15,16]. Cross-kingdom studies on plant-based miRNAs similarly demonstrate that only high-copy molecules reach the concentration threshold required to effectively suppress target gene expression in the consumer. Most early studies focused on qualitative miRNA composition—mere categorized as "presence or absence" which frequently led to an overestimation of the biological effect of rare, low-abundance molecules. These considerations highlight the necessity of quantitative frameworks, such as our Global Score, to estimate actual regulatory impact.

In this context, the observed contraction of the dominant miRNA pool—from 35 species in humans to just 12 in camels—reflects divergent evolutionary priorities centered on dosage and precision. Human milk's relative diversification functions as a "regulatory symphony," where the regulatory weight is distributed across a broader repertoire to manage the complex neurogenesis and multifaceted immune programming unique to human ontogeny. Conversely, the extreme concentration identified in camel milk, where approximately 62% of the total pool consists of a single molecule (miR-148a), suggests a "biological intervention" strategy. This intensive focus on high-copy molecules targeting key nodes, such as the STAT3/Th17 axis and PPARD signaling, explains the potent immunometabolic properties attributed to camel milk. These findings suggest that while human and bovine milk provide a broad homeostatic blueprint, camel milk may act more as a specialized tool for correcting inflammatory and metabolic states than as a general nutritional source.

In our study, the Global Score metric addresses this gap by integrating quantitative abundance (read counts) with thermodynamic binding affinity (miRWalk score). Crucially, we cross-validated these drivers using the mirDIP 5.2 database, identifying robust regulatory nodes such as RIMS2, STAT3, and NLGN3. By requiring targets to meet both miRWalk's energy criteria and mirDIP's multi-algorithm consensus, we identify drivers that carry both theoretical binding capacity and a substantial quantitative advantage in milk. This dual-database approach strengthens the robustness of the analysis in the context of ongoing debate on dietary nucleic acid bioavailability.

The universal cumulative weighting approach was briefly explored previously, [7,17], and our implementation draws on methodology related to that of Vittori et al. [4]. Within this methodological framework, the comparative analysis of the three milk types provides insights into the potential biological implications of these regulatory patterns. The integrated comparative analysis confirms a distinct hierarchy of regulatory power across GO, KEGG, and Hallmark signatures. Over 80% of target genes are common to all three milks, yet the species-specific fractions carry clinical significance. Human milk remains the 'gold standard' for miRNA-mediated regulation of infant immune development and cognitive programming [18], while bovine milk demonstrates significant overlap with the human "epigenetic template." The fact that 14.8% of genes are shared exclusively between bovine and human milk (e.g., the validated nuclear transport node XPO1), versus only 1.5% for human-camel, underscores that bovine milk is a functionally closer analogue to human milk.

Importantly, camel milk's unique regulatory fraction exerts a highly specific influence. The targeted modulation of PPARD-mediated fatty acid metabolism and the JAK-STAT pathway via STAT3 provides the first miRNA-level mechanistic basis for its clinically observed anti-inflammatory and anti-diabetic effects. The high consensus score for STAT3 in our integrated analysis suggests that this interaction is a reliable therapeutic target of camel milk exosomes. STAT3 signaling is essential for initiating the Th17 program; its inhibition can switch the balance from pro-inflammatory Th17 toward anti-inflammatory Treg cells, aligning with the "metabolic corrector" profile often attributed to camel milk.

Cross-species synergies reveal non-obvious functional bridges: Cow_Camel jointly regulate cellular energetics (TCA cycle via DLAT), while Human_Cow synergy is directed toward genomic stability (DNA replication and mismatch repair).

Future research should determine the specific milk concentrations required to trigger these specialized functions and characterize the molecular gastrointestinal environment that optimizes exosome preservation.

The analysis was restricted to miRNA-gene interactions with a miRWalk score > 0.95 in the 3'UTR region and mirDIP classification within "High" and "Very High" confidence classes. While these criteria ensure high specificity and statistical robustness, they may exclude valid targets regulated under broader parameters. The reliance on published sequencing datasets and computational prediction necessitates further experimental validation, particularly in vitro and in vivo confirmation of STAT3 and NLGN3 regulation by milk-specific miRNA pools.

5. Conclusions

Milk miRNAs function as systemic epigenetic regulators, employing distinct species-specific strategies. While a conserved core (Common All) ensures universal developmental stability through neuroplasticity and MAPK/Ras growth signaling, species-specific signatures reflect divergent physiological priorities. Human milk maintains a broad regulatory for complex neuro-immune development, whereas camel milk implements a "biological intervention" strategy, utilizing high-copy miRNAs to intensively target immunometabolic nodes like STAT3 and PPARD. Notably, bovine milk provides a closer functional analog to the human epigenetic template (14.8% overlap) than camel milk. These findings establish a molecular framework for the unique therapeutic properties of camel milk and provide a quantitative basis for its application in personalized nutrition and exosome-based biomedical research

Supplementary Materials: All dataset used in this study can be downloaded at: https://doi.org/10.5281/zenodo.19115625.

Author Contributions: Conceptualization, V.C and M.Z; methodology, M.Z.; software, M.Z.; validation, M.C.C., E.C. and G.C..; formal analysis, Amine Akouya; investigation, A.D.D; resources, M.C.C.; data curation, K.D.; writing original draft preparation, M.Z.; writing review and editing, A.A.; visualization, D.K.; supervision, M.A.J. and V.C.; project administration, N.Y.S.; funding acquisition, M.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: The study did not require ethical approval, because it did not had direct contact with human and animal subjects. The study was solely based on publicly available datasets.

Data Availability Statement: Data used in this study can be obtained from corresponding author upon request.

Acknowledgments: This article was made possible by the Mir-Nat Global Health network (https://www.mir-nat.com/), and in particular by the bioinformatics studies of Dr. Stefano Pirrò, the University of Rome Tor Vergata, which developed the necessary biological expertise, and the numerous students of the Faculty of Medicine at the Good Samaritan University Hospital in N'Djamena, Chad, who are studying camel habits for improved milk production. Daha Hussein and Shafii Mohamed from Centre for Camel Research and Development, Faculty of Veterinary Medicine and Animal Husbandry, Somali National University, Mogadishu, Somalia, for assembling Camel/Camelid peer reviewed scientific publications on health, production systems, microbiome, welfare, and climate resilience and participating conceptualization discussion meeting for the study, respectively. Professor Hassan Hassan, Dean Faculty of Veterinary Medicine and Animal Husbandry, Somali National University, for participating conceptualization discussion meeting for the study, and for his significant contributions to camel research in Somalia.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Carrillo-Lozano E, Sebastián-Valles F, Knott-Torcal C. Circulating microRNAs in Breast Milk and Their Potential Impact on the Infant. Nutrients. 2020 Oct 8;12(10):3066. doi: 10.3390/nu12103066. PMID: 33049923; PMCID: PMC7601398.
2. Lin Zhang, Ting Chen, Yulong Yin, Chen-Yu Zhang, Yong-Liang Zhang, Dietary microRNA—A Novel Functional Component of Food, Advances in Nutrition, Volume 10, Issue 4, 2019, Pages 711-721, ISSN 2161-8313, https://doi.org/10.1093/advances/nmy127.
3. Ngu A, Wang S, Wang H, Khanam A, Zempleni J. Milk exosomes in nutrition and drug delivery. Am J Physiol Cell Physiol. 2022 May 1;322(5):C865-C874. doi: 10.1152/ajpcell.00029.2022. Epub 2022 Mar 23. PMID: 35319899; PMCID: PMC9037700.
4. Ari Yuka S, Yilmaz A. Network based multifactorial modelling of miRNA-target interactions. PeerJ. 2021 Mar 19;9:e11121. doi: 10.7717/peerj.11121. PMID: 33777541; PMCID: PMC7983860.
5. Kleinjan M, van Herwijnen MJ, Libregts SF, van Neerven RJ, Feitsma AL, Wauben MH. Regular Industrial Processing of Bovine Milk Impacts the Integrity and Molecular Composition of Extracellular Vesicles. J Nutr. 2021 Jun 1;151(6):1416-1425. doi: 10.1093/jn/nxab031. PMID: 33768229.

6. Mullokandov G, Baccarini A, Ruzo A, Jayaprakash AD, Tung N, Israelow B, Evans MJ, Sachidanandam R, Brown BD. High-throughput assessment of microRNA activity and function using microRNA sensor and decoy libraries. Nat Methods. 2012 Jul 1;9(8):840-6. doi: 10.1038/nmeth.2078. PMID: 22751203; PMCID: PMC3518396.
7. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF, Pogosova-Agadjanyan EL, Stirewalt DL, Tait JF, Tewari M. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci U S A. 2011 Mar 22;108(12):5003-8. doi: 10.1073/pnas.1019055108. Epub 2011 Mar 7. PMID: 21383194; PMCID: PMC3064324.
8. Wu R, Yue C, Xie Y, Fu Y, Yu X, Li Z, Yang B. Sequencing and analysis of micro RNAs in camel milk exosomes. Acta Vet Hung. 2023 Sep 19. doi: 10.1556/004.2023.00869. Epub ahead of print. PMID: 37725504.
9. van Herwijnen MJC, Driedonks TAP, Snoek BL, Kroon AMT, Kleinjan M, Jorritsma R, Pieterse CMJ, Hoen ENMN, Wauben MHM. Abundantly Present miRNAs in Milk-Derived Extracellular Vesicles Are Conserved Between Mammals. Front Nutr. 2018 Sep 18;5:81. doi: 10.3389/fnut.2018.00081. PMID: 30280098; PMCID: PMC6153340.
10. Sticht C, De La Torre C, Parveen A, Gretz N.: miRWalk: An online resource for prediction of microRNA binding sites. PLoS One. 2018 Oct 18;13(10):
11. Tokar T, Pastrello C, Rossos AEM, Abovsky M, Hauschild AC, Tsay M, Lu R, Jurisica I. mirDIP 4.1-integrative database of human microRNA target predictions. Nucleic Acids Res. 2018 Jan 4;46(D1):D360-D370. doi: 10.1093/nar/gkx1144. PubMed PMID: 29194489; PubMed Central PMCID: PMC5753284
12. Zempleni J, Baier SR, Howard KM, Cui J. Gene regulation by dietary microRNAs. Can J Physiol Pharmacol. 2015 Dec;93(12):1097-102. doi: 10.1139/cjpp-2014-0392. Epub 2015 Apr 14. PMID: 26222444; PMCID: PMC4743494.
13. Wolf T, Baier SR, Zempleni J. The Intestinal Transport of Bovine Milk Exosomes Is Mediated by Endocytosis in Human Colon Carcinoma Caco-2 Cells and Rat Small Intestinal IEC-6 Cells. J Nutr. 2015 Oct;145(10):2201-6. doi: 10.3945/jn.115.218586. Epub 2015 Aug 12. PMID: 26269243; PMCID: PMC4580964.
14. Zempleni, J. Milk exosomes: beyond dietary microRNAs. *Genes Nutr* 12, 12 (2017). https://doi.org/10.1186/s12263-017-0562-6
15. Melnik, B.C.; Stremmel, W.; Weiskirchen, R.; John, S.M.; Schmitz, G. Exosome-Derived MicroRNAs of Human Milk and Their Effects on Infant Health and Development. *Biomolecules* 2021, *11*, 851. https://doi.org/10.3390/biom11060851
16. Mullokandov G, Baccarini A, Ruzo A, Jayaprakash AD, Tung N, Israelow B, Evans MJ, Sachidanandam R, Brown BD. High-throughput assessment of microRNA activity and function using microRNA sensor and decoy libraries. Nat Methods. 2012 Jul 1;9(8):840-6. doi: 10.1038/nmeth.2078. PMID: 22751203; PMCID: PMC3518396.
17. Zempleni J, Aguilar-Lozano A, Sadri M, Sukreet S, Manca S, Wu D, Zhou F, Mutai E. Biological Activities of Extracellular Vesicles and Their Cargos from Bovine and Human Milk in Humans and Implications for Infants. J Nutr. 2017 Jan;147(1):3-10. doi: 10.3945/jn.116.238949. Epub 2016 Nov 16. PMID: 27852870; PMCID: PMC5177735.
18. Arain MA, Salman HM, Ali M, Khaskheli GB, Barham GS, Marghazani IB, Ahmed S. A Review on Camel Milk Composition, Techno-Functional Properties and Processing Constraints. Food Sci Anim Resour. 2024 Jul;44(4):739-757. doi: 10.5851/kosfa.2023.e18. Epub 2024 Jul 1. PMID: 38974725; PMCID: PMC11222694.