Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Soy Isoflavone Improves Reproductive Performance and Antioxidant Capacity of Sows and Reshapes Colostrum-Derived Exosomal microRNA Profiles

Qiming Duan
Sichuan Agricultural University
Xiang Li
Sichuan Agricultural University
Yan Li
Guangxi Normal University
Kunhong Xie
Sichuan Agricultural University
Jun Li
Guangxi Normal University
Yuheng Luo
Sichuan Agricultural University
Ping Zheng
Sichuan Agricultural University
Xiangbing Mao
Sichuan Agricultural University
Hui Yan
Sichuan Agricultural University
Jun He
hejun8067@163.com
Sichuan Agricultural University

Research Article

Keywords: Soy isoflavone, Sow, Reproductive performance, Colostrum-derived exosomes, microRNA sequencing

Posted Date: May 5th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9332396/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

This study investigated the effects of dietary soy isoflavone supplementation on reproductive performance, antioxidant capacity, and colostrum-derived exosomal microRNA profiles in sows, with the aim of exploring the molecular basis of maternal–offspring integrated regulation. A total of 120 Landrace × Yorkshire sows were assigned to either a control diet or a diet supplemented with 200 mg/kg soy isoflavone from gestation day 106 to lactation day 28. Reproductive performance and serum antioxidant indices were evaluated, and colostrum-derived exosomes were isolated for small RNA sequencing and bioinformatic analysis.

Results

Dietary soy isoflavone supplementation significantly increased the total number of piglets born, the number of live-born piglets, litter weight at birth, and litter weight at weaning, while shortening farrowing duration ($P < 0.05$). In addition, soy isoflavone significantly elevated serum total antioxidant capacity and catalase activity on lactation day 21 ($P < 0.05$). Transmission electron microscopy, nanoparticle tracking analysis, and flow cytometry confirmed the successful isolation of colostrum-derived exosomes. Small RNA sequencing showed that most microRNAs ranged from 18 to 26 nucleotides, with a predominant peak at 22–23 nucleotides. Principal component analysis and differential expression analysis revealed that soy isoflavone markedly reshaped the microRNA cargo of colostrum-derived exosomes. Functional enrichment analysis indicated that the predicted target genes of differentially expressed microRNAs were mainly involved in transcriptional regulation, kinase-mediated signaling, inflammatory responses, and metabolic pathways, including mitogen-activated protein kinase, Ras, Rap1, endocytosis, autophagy, and adherens junction pathways. Integrated network analyses further suggested coordinated regulation of inflammatory signaling and metabolic homeostasis.

Conclusions

Dietary soy isoflavone supplementation improved sow reproductive performance and antioxidant capacity, while reshaping colostrum-derived exosomal microRNA profiles and their associated regulatory networks. These findings provide a potential molecular basis for maternal–offspring integrated regulation.

Introduction

Reproductive efficiency of sows is a key determinant of productivity and profitability in modern swine production systems. Improving litter size, piglet viability, and early growth performance remains a major objective in nutritional management strategies. Maternal nutrition during late gestation and lactation plays a critical role in determining reproductive outcomes as well as neonatal development. In addition to influencing fetal growth and parturition, maternal nutritional status affects milk composition and the transfer of bioactive components to piglets [1, 2]. Therefore, exploring functional dietary interventions that enhance sow reproductive performance while supporting offspring health is of considerable importance in animal nutrition.

Soy isoflavones (SIF) are naturally occurring phytoestrogens widely present in soybean and soybean-derived feed ingredients, which constitute a major protein source in swine diets [3]. Therefore, SIF have practical relevance in commercial pig production. Structurally similar to endogenous estrogens, SIF can bind to estrogen receptors and modulate multiple signaling pathways associated with reproduction, growth, oxidative stress, and immune responses [4]. In sow production systems, late gestation and lactation represent physiologically demanding stages characterized by elevated oxidative stress and increased metabolic burden, which may lead to prolonged farrowing duration, reduced piglet vitality, and impaired lactation performance [5, 6]. Previous studies have demonstrated that dietary SIF supplementation improves antioxidant status in sows and growing pigs by enhancing total antioxidant capacity and antioxidant enzyme activities, while also modulating inflammatory cytokine expression and alleviating oxidative damage [7, 8]. In various animal models, phytoestrogens have been reported to influence ovarian function, improve uterine environment, and optimize parturition processes, thereby increasing litter size and offspring viability [9]. Within the framework of maternal–offspring integrated regulation, maternal nutritional status not only affects the physiological condition of the sow but also influences neonatal development through the transfer of bioactive components via the placenta and milk [10]. Evidence suggests

that supplementation of functional plant-derived compounds can alter milk composition, including immunoglobulins, cytokines, and antioxidant-related factors, subsequently improving antioxidant capacity and immune function in offspring [10]. Therefore, SIF may exert dual regulatory effects: directly modulating maternal reproductive physiology and oxidative status, and indirectly influencing offspring development by altering the bioactive composition of colostrum and milk.

Milk-derived extracellular vesicles, particularly exosomes, have recently been recognized as important mediators of maternal-offspring communication [11]. Exosomes are nano-sized (30–150 nm) membrane-bound vesicles present in biological fluids, including colostrum, and carry diverse molecular cargo such as proteins, lipids, messenger RNAs, and microRNAs (miRNAs) [12]. Due to their lipid bilayer structure, exosomes protect their RNA contents from enzymatic degradation, enabling stable transfer of regulatory molecules to recipient cells [13]. Increasing evidence suggests that milk-derived exosomes can survive gastrointestinal conditions and be internalized by intestinal epithelial cells, thereby influencing gene expression and cellular signaling in neonates [14]. Among exosomal cargos, miRNAs are small non-coding RNAs (18–26 nucleotides) that regulate gene expression at the post-transcriptional level by binding to target mRNAs [15]. A single miRNA can regulate multiple genes, forming complex regulatory networks involved in immune responses, inflammatory signaling, and metabolic processes [16]. The stability of miRNAs within exosomes further supports their potential functional role in early-life development [17]. However, the regulatory profile of colostrum-derived exosomal miRNAs under maternal dietary modulation remains largely unexplored in swine.

Therefore, the present study aimed to evaluate the effects of dietary soy isoflavone supplementation on reproductive performance and antioxidant capacity of sows, to characterize colostrum-derived exosomal miRNA profiles, and to construct integrated regulatory networks to explore potential molecular mechanisms. By combining physiological parameters with high-throughput sequencing and bioinformatics analyses, this study sought to provide a comprehensive understanding of how maternal SIF supplementation influences reproductive outcomes and colostrum-derived molecular signals within the framework of maternal-offspring integrated regulation.

Material and Method

Experimental design and diet

This study was carried out at a commercial pig farm of Luoshi Animal Husbandry Co., Ltd. in Wangcang County, Guangyuan City, Sichuan Province, China. All experimental Landrace × Yorkshire sows were obtained from this farm and were maintained under standard farm management and feeding conditions throughout the study period. A total of 120 Landrace × Yorkshire (LY) sows at day 106 of gestation were enrolled in a single-factor design. Based on similar parity (3–5) and backfat thickness, sows were randomly assigned to either a control diet (CON) or a soy isoflavone-supplemented diet (SIF; 200 mg/kg), with 60 sows per treatment (one sow per replicate). The trial lasted from gestation day 106 to lactation day 28 (weaning). The basal diet was a corn-soybean meal diet formulated to meet nutrient requirements for lactating sows (NRC, 2012) [18] with balanced vitamins and minerals (Table 1). In the SIF group, soy isoflavones replaced an equal amount of corn. To ensure homogeneity, SIF was first mixed with the vitamin–mineral premix and then blended with the remaining ingredients; all diets were pelleted. Soy isoflavones were derived from non-GMO soybean (total soybean isoflavones ≥ 10%; Guilin Layn Natural Ingredients Corp., China).

Table 1
Composition and nutrient levels of basic rations

Item	Content (%)
Ingredients	
Corn	56.00
Soybean meal	25.00
Wheat bran	7.00
Soybean oil	4.00
Fish meal	2.00
L-Lysine HCl	0.50
Limestone	1.50
Dicalcium phosphate	1.50
NaCl	0.50
Vitamin–mineral premix[1]	2.00
Total	**100.00**
Nutrient levels[2]	
DE (MJ/kg)	13.41
Crude protein (%)	17.50
Lysine (%)	1.34
Calcium (%)	1.10
Total phosphorus (%)	0.80
Available phosphorus (%)	0.58

[1] The premix provides the following per kilogram of diet: Vitamin A 11000 IU; Vitamin D 20000 IU; Vitamin E 44.09 IU; Vitamin K 4.4 mg; Vitamin B2 1.1 mg; Vitamin B6 15.2 mg; Vitamin B12 25 μg; Nicotinic acid 55.1 mg; Pantothenic acid 33 mg; Choline 1551 mg; Biotin 0.22 mg; Folic acid 1.7 mg; Zn 120.3 mg; Mn 39.7 mg; Fe 100.0 mg; Cu 20.0 mg; I 3.0 mg; Se 3.0 mg.

[2] Nutritional levels of the formula are calculated values.

Sample collection

Sows were moved to a thoroughly disinfected farrowing facility at gestation day 104 and housed individually in farrowing crates on fully slatted floors; room temperature was maintained at 19–25°C. Experimental diets were provided from gestation day 106. Feed allowance was reduced by 0.5 kg/day during the three days pre-farrowing and was withheld on the farrowing day. Sows were fed three times daily (08:00, 14:30, 20:30). After farrowing, sows received 1.0 kg on lactation day 1 and were increased by 0.5-1.0 kg/day to ad libitum intake within one week.

For milk sampling, colostrum (within 1 h postpartum) and mature milk (lactation day 14) were collected from six healthy sows per treatment matched for parity and backfat thickness. Approximately 20 mL milk was collected per sow, evenly from the anterior, middle, and posterior teats on the same side after cleaning the teat area with alcohol wipes. For mature milk collection (day 14), oxytocin was injected via the ear vein to facilitate milk let-down. Samples were stored at -20°C until analysis.

For blood sampling, six healthy sows per treatment were sampled at 08:00 on lactation days 1, 14, and 21 (fasted). Approximately 5 mL of blood was obtained from the anterior vena cava and placed into collection tubes. Following coagulation at room temperature

for 30 min, samples were centrifuged at 3500 r/min for 10 min, after which serum was separated, aliquoted, and stored at − 20°C.

No euthanasia or sacrifice was performed in this study, as only blood and milk samples were collected from live sows. No terminal procedures, general anaesthesia, or euthanasia agents were used. Blood collection from the anterior vena cava and milk sampling were brief routine procedures performed by trained personnel with appropriate manual restraint to minimize animal stress and discomfort.

Growth Performance of Suckling Piglets

To minimize variation among litters, piglets were redistributed after farrowing within each treatment group so that each sow nursed 12 piglets with similar initial birth weights. Piglet health status and nursing conditions were monitored daily. Piglets started creep feeding at day 14. Piglets were weighed at 08:00 after litter equalization and again on lactation day 28 to calculate suckling growth performance.

Colostrum and Mature Milk Composition

Milk fat, crude protein, true protein, lactose, total solids, solids-not-fat, and somatic cell count were analyzed by a certified testing laboratory (Qingdao Kechuang Quality Testing Co., Ltd., China). Milk immunoglobulins (IgA, IgG, IgM) were determined using commercial ELISA kits (Jiangsu Enzyme Immunity Industry Co., Ltd., China) following the manufacturer's instructions.

Serum Hormones

Serum concentrations of estradiol (E2; MM-0480O1), progesterone (PROG; MM-1205O1), leptin (LEP; MM-0395O1), and prolactin (PRL; MM-0907O1) were measured using commercial ELISA kits (Jiangsu Enzyme Immunity Industry Co., Ltd., China) according to the manufacturer's protocols.

Serum proinflammatory cytokines and immunoglobulin detection

Serum immunoglobulins (IgA, IgG, IgM) were quantified using commercial ELISA kits (Jiangsu Enzyme Immunity Industry Co., Ltd., China) following the manufacturer's instructions. Proinflammatory cytokines were not assessed in this lactation experiment.

Serum Antioxidant Capacity

Commercial kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) were used to determine serum total antioxidant capacity, catalase, malondialdehyde, superoxide dismutase, and glutathione according to the manufacturers' instructions.

Serum Immunoglobulins

Serum IgA, IgG, and IgM concentrations were measured by ELISA (Jiangsu Enzyme Immunity Industry Co., Ltd., China) following the manufacturer's protocols.

Small RNA library preparation and sequencing

Small RNA libraries were prepared from total RNA using the TruSeq Small RNA Sample Preparation protocol (Illumina). Briefly, 5 μg total RNA was used as input, and 3' and 5' RNA adapters were sequentially ligated to enrich for small RNAs. For 3' adapter ligation, RNA was first denatured at 70°C for 2 min and immediately chilled on ice, followed by ligation with T4 RNA Ligase 2 (deletion mutant) in ligation buffer at 28°C for 1 h; reactions were terminated with stop solution and further incubated at 28°C for 15 min. For 5' adapter ligation, the 5' adapter was pre-heated at 70°C for 2 min and chilled on ice, then ligated to the 3'-ligated products in the presence of ATP and T4 RNA ligase at 28°C for 1 h. Adapter-ligated RNAs were reverse-transcribed using an RNA RT primer and SuperScript II reverse transcriptase (denaturation 70°C for 2 min, followed by reverse transcription at 50°C for 1 h), and cDNA libraries were amplified by indexed PCR (PCR mix plus RP1 and index primers; 11 cycles of 98°C 30 s/60°C 30s/72°C 30 s, with a

final extension at 72°C for 10 min). Amplified libraries were size-selected by 6% TBE-PAGE (145 V, ~ 60 min), and bands corresponding to adapter-ligated small RNAs (approximately 145–160 bp, representing ~ 22–30 nt inserts) were excised, eluted in nuclease-free water with agitation (≥ 2 h; overnight if needed), and recovered by filtration; ethanol precipitation was performed when higher concentration was required. Final library size distribution and concentration were assessed using an Agilent 2100 Bioanalyzer (High Sensitivity DNA chip). Libraries were diluted/denatured according to Illumina recommendations prior to cluster generation and sequencing. Libraries were sequenced on the Illumina sequencing platform by LC-BIO Co., Ltd (HangZhou, China).

Bioinformatic analysis of small RNA sequencing data

Raw sequencing reads were processed using the ACGT101-miR pipeline (LC Sciences, Houston, TX, USA). Briefly, adaptor dimers, low-quality reads, junk sequences, low-complexity reads, common non-coding RNAs (including rRNA, tRNA, snRNA, and snoRNA), and repetitive sequences were removed. After trimming the 3' adaptor sequences, clean reads with lengths of 18–26 nt were retained for downstream analysis.

To identify known miRNAs, the clean reads were aligned to porcine precursor miRNAs deposited in miRBase v22.0 using BLAST. During alignment, terminal length variation at both the 3' and 5' ends and up to one internal mismatch were permitted. Reads mapped to annotated mature miRNAs on the known hairpin arms were classified as known miRNAs, whereas reads mapped to the opposite arm of known precursor hairpins were regarded as novel 5p- or 3p-derived miRNA candidates.

The remaining unmapped reads were subsequently aligned to precursor miRNAs from other selected species in miRBase v22.0 and then mapped to the Sus scrofa reference genome (Sscrofa11.1) to determine their genomic loci. Reads that could not be assigned to known miRNAs were further subjected to novel miRNA prediction. Putative novel miRNAs were identified based on genomic mapping and secondary structure prediction using RNAfold, according to predefined criteria including hairpin length, loop length, bulge size, stem pairing, and minimum folding free energy.

Statistical Analysis

Raw data were first organized and calculated in Microsoft Excel 2021. Statistical analyses were performed using SPSS Statistics 27.0 (IBM, Armonk, NY, USA). Outliers were screened by exploratory data analysis, and data normality was assessed using the Shapiro - Wilk test. Variables that met the assumption of normality were analyzed using an independent-samples t - test to compare the CON and SIF groups. For sow reproductive performance traits, the litter was considered the experimental unit; for all other variables, the individual sow (randomly selected within each treatment) was used as the experimental unit. Values are reported as mean accompanied by the SEM. Statistical significance was assigned at $P < 0.05$, strong significance at $P < 0.01$, whereas $0.05 \leq P < 0.10$ was considered to indicate a statistical trend.

For small RNA sequencing analysis, miRNA abundance was normalized as counts per million (CPM) to account for differences in library size among samples. Only miRNAs with detectable expression (CPM > 0 in at least 50% of samples) were retained for further analysis. Differential expression analysis between the control (CON) and soy isoflavone-supplemented (SIF) groups was performed using normalized read counts generated from the ACGT101-miR pipeline, and miRNAs with $P < 0.05$ were considered differentially expressed.

Principal component analysis (PCA), Pearson correlation analysis, and hierarchical clustering were conducted to evaluate sample reproducibility and global expression patterns of colostrum-derived exosomal miRNAs. Expression profiles were visualized using heatmaps and volcano plots. Predicted target genes of differentially expressed miRNAs were further subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. Enrichment significance was assessed using Fisher's exact test, and $P < 0.05$ was considered statistically significant.

Results

Dietary soybean isoflavones supplementation improves the reproductive performance of sows

As shown in Table 2, Dietary soy isoflavones supplementation significantly elevated the total and the live-born numbers of piglets born per litter ($P < 0.05$). Meanwhile, dietary soy isoflavones supplementation significantly increased the litter weight at birth and the

litter weight at weaning of piglets ($P < 0.05$). Moreover, compared to the CON group, we found that a shorter farrowing duration of sows in the SIF group ($P < 0.05$).

Table 2
Effects of dietary soybean isoflavones on performance of sows

Item	CON	SIF	SEM	*P*-value
Total number of piglets born per litter	11.57[b]	12.22[a]	0.325	< 0.05
Number of live-born piglets per litter	10.42[b]	11.10[a]	0.340	< 0.05
Number of weak-born piglets per litter	0.70	0.60	0.050	0.387
Number of mummified fetuses and stillborn piglets per litter	1.15	1.12	0.015	0.182
Total live-born litter weight (kg)	14.04[b]	14.89[a]	0.110	< 0.05
Average birth weight of live-born piglets (kg)	1.22	1.22	0.001	0.683
Farrowing duration (min)	172.4[a]	156.6[b]	7.900	0.023
Litter weight at weaning (kg)	97.00[b]	102.93[a]	0.966	< 0.05
Average weaning weight (kg)	8.40	8.42	0.054	0.80

Note: The results are expressed by mean and total standard error; Within each row, the use of distinct lowercase letters 'a' and 'b' signifies statistically significant variations ($P < 0.05$), while a trend is inferred when $0.05 \leq P < 0.10$.

Dietary soy isoflavones enhances the serum antioxidant capacity of lactating sows

The related parameters of serum were listed in Table 3. Soy isoflavones supplementation significantly elevated the serum total antioxidant capacity and the catalase activity during the day 21 of lactation ($P < 0.05$).

Table 3
Effects of dietary soy isoflavones on serum hormones, antioxidant capacity and immunoglobulin of lactating sows

Item	CON	SIF	SEM	*P*-value
Sow serum (Lactation Day 0)				
Estradiol (E2), pmol/L	109.3	116.8	2.53	0.15
Progesterone (P), pmol/L	2258	2168	71.64	0.55
Prolactin (PRL), ng/L	252.9	247.1	7.58	0.72
Leptin (LEP), ng/L	2033	2087	61.93	0.69
Total antioxidant capacity (T-AOC), U/mL	1.26	1.74	0.18	0.18
Superoxide dismutase (SOD), U/mL	319.3	378.9	34.01	0.41
Catalase (CAT), U/mL	9.77	12.39	0.96	0.19
Glutathione peroxidase (GSH-Px), U/mL	2092	2263	55.11	0.13
Malondialdehyde (MDA), nmol/mL	7.51	4.65	2.06	0.40
Immunoglobulin A (IgA), μg/mL	32.57	29.95	1.26	0.32
Immunoglobulin G (IgG), μg/mL	374.9	393.6	12.95	0.50
Immunoglobulin M (IgM), μg/mL	35.37	32.54	1.14	0.23
Sow serum (Lactation Day 14)				
Estradiol (E2), pmol/L	117	116.4	2.46	0.91
Progesterone (P), pmol/L	2007	2047	49.41	0.71
Prolactin (PRL), ng/L	219.4	222	5.67	0.83
Leptin (LEP), ng/L	1617	1684	52.19	0.55
Total antioxidant capacity (T-AOC), U/mL	1.15	1.90	0.24	0.11
Superoxide dismutase (SOD), U/mL	233.4	254.7	22.64	0.66
Catalase (CAT), U/mL	8.14	10.34	0.87	0.33
Glutathione peroxidase (GSH-Px), U/mL	2178	2189	118.75	0.96
Malondialdehyde (MDA), nmol/mL	5.43	3.87	0.67	0.26
Immunoglobulin A (IgA), μg/mL	33.71	35.65	0.95	0.33
Immunoglobulin G (IgG), μg/mL	384.2	400.8	12.05	0.52
Immunoglobulin M (IgM), μg/mL	41.75	43.03	1.67	0.72
Sow serum (Lactation Day 21)				
Estradiol (E2), pmol/L	112.5	116.4	3.07	0.58
Progesterone (P), pmol/L	1677	1653	61.36	0.85
Prolactin (PRL), ng/L	236.3	234.8	6.62	0.92
Leptin (LEP), ng/L	1835	1753	48.50	0.42
Total antioxidant capacity (T-AOC), U/mL	0.91^{b}	1.52^{a}	0.14	0.02
Superoxide dismutase (SOD), U/mL	290	321.9	15.37	0.32
Catalase (CAT), U/mL	7.77^{b}	14.51^{a}	1.45	0.01

Item	CON	SIF	SEM	*P*-value
Glutathione peroxidase (GSH-Px), U/mL	1715	1665	73.33	0.75
Malondialdehyde (MDA), nmol/mL	4.60	3.25	0.48	0.17
Immunoglobulin A (IgA), μg/mL	32.37	33.52	0.93	0.56
Immunoglobulin G (IgG), μg/mL	366.2	373.4	9.49	0.73
Immunoglobulin M (IgM), μg/mL	41.58	44.06	1.32	0.37

Isolation and characterization of colostrum-derived exosomes and small RNA profiling

Transmission electron microscopy (TEM) revealed that the isolated vesicles exhibited typical cup-shaped or spherical morphology with uniform size distribution, consistent with the structural characteristics of exosomes (Fig. 1A, B). Nanoparticle tracking analysis (NTA) demonstrated that the particle sizes were mainly distributed within the range of 30–150 nm. The average particle size was 83.48 nm in the CON group and 83.88 nm in the SIF group, indicating comparable size distributions between the two groups (Fig. 1A, B). Flow cytometry analysis showed nearly 100% positivity in both groups, confirming successful isolation and high purity of exosomes. A summary of the miRNA-seq results for sow colostrum samples is provided in Table 4. Across the eight constructed transcriptome libraries, each sample generated between 18,231,049 and 23,462,818 raw reads. After quality control and filtering, 7,562,561 to 15,760,164 clean reads per sample were successfully mapped to the annotated porcine reference genome (Sus_scrofa.Sscrofa11.1.dna.toplevel.fa.gz). The number of valid reads ranged from 91,355 to 183,399 for each sample, with uniquely mapped valid reads accounting for an average of 43.07%. Analysis of small RNA length distribution showed that most miRNAs were 18–26 nucleotides in length, with the dominant peak located at 22–23 nt (Fig. 1C, D). This pattern agrees with the characteristic size of mature miRNAs, indicating that the small RNA libraries were of high quality.

(A–B) Transmission electron microscopy (TEM) images of exosomes isolated from sow colostrum in the CON and SIF groups, showing typical cup-shaped or spherical vesicular morphology. Nanoparticle tracking analysis (NTA) showed that the particle sizes were mainly distributed within the range of 30–150 nm. Flow cytometry analysis indicated a high proportion of positive exosomal particles in both groups.

1. (C) Length distribution of small RNA sequencing reads across all samples. The majority of small RNAs were distributed between 18 and 26 nt.
2. (D) Distribution of unique miRNA counts according to sequence length, with a predominant peak at 22–23 nt, consistent with the typical length of mature miRNAs.

Table 4
Summary of data obtained from RNA-Seq of sow ovaries.

Sample*	CON_1	CON_2	CON_3	CON_4	SIF_1	SIF_2	SIF_3	SIF_4
Raw reads	19,571,879	23,462,818	20,611,797	19,753,816	20,252,685	19,662,714	18,231,049	19,681,433
3ADT&length filter	6,624,399	7,630,018	4,205,762	12,151,869	9,949,387	4,838,761	6,158,494	6,398,083
% of 3ADT&length filter	33.85	32.52	20.40	61.52	49.13	24.61	33.78	32.51
% of uniquely 3ADT&length filter	52.99	52.89	55.12	56.57	53.76	52.01	53.41	47.17
Junk reads	38757	72636	84725	39386	52612	103634	85092	92972
% of Junk reads	0.20	0.31	0.41	0.20	0.26	0.53	0.47	0.47
% of uniquely Junk reads	0.99	1.00	0.95	1.01	0.97	0.81	0.98	1.01
Clean reads	12,908,723	15,760,164	16,321,310	7,562,561	10,250,686	14,720,319	11,987,463	13,190,378
% of Clean reads	65.96	67.17	79.18	38.28	50.61	74.86	65.75	67.02
% of uniquely Clean reads	52.00	51.88	54.17	55.56	52.80	51.21	52.44	46.16
Rfam	565,733	964,658	775,885	614,195	718,537	863,905	746,982	983,282
% of Rfam	2.89	4.11	3.76	3.11	3.55	4.39	4.10	5.00
% of uniquely Rfam	1.21	1.39	1.25	1.09	1.24	1.39	1.20	1.24
mRNA	565,733	964,658	775,885	614,195	718,537	863,905	746,982	983,282
% of mRNA	55.09	53.79	63.04	30.25	39.94	54.80	49.63	51.31
% of uniquely mRNA	2.73	2.41	2.27	1.92	2.05	1.60	2.13	2.30
Repeats	10,782,367	12,619,697	12,993,905	5,975,988	8,088,251	10,775,077	9,048,918	10,098,075
% of Repeats	0.47	0.59	0.66	0.87	0.91	0.53	0.70	0.63
% of uniquely Repeats	0.18	0.18	0.19	0.13	0.15	0.13	0.18	0.18
valid reads	91,355	138,443	135,841	172,618	183,399	103,955	126,965	123,097
% of reads	10.09	12.03	14.75	7.23	9.46	18.19	14.54	14.03
% of uniquely reads	42.60	42.84	40.90	39.83	42.40	44.53	42.74	48.72

CON, basal diet; SIF, the basal diet supplemented with 150 mg/kg soybean isoflavones.

* The statistics are presented for each sample separately.

3ADT&length filter: reads removed due to 3ADT not found and length with < 18 nt and > 25 nt were removed(for plants); length with < 18 and > 26 were remove(for animals).

Junk reads: Junk: >=2N, >=7A, >=8C, >=6G, >=7T, >=10Dimer, >=6Trimer, or > = 5Tetramer.

Clean reads: equal to raw reads – 3ADT&length filter - Junk reads.

Rfam: Collection of many common non-coding RNA families except micro RNA.

Repeats: Prototypic sequences representing repetitive DNA from different eukaryotic species

Soy isoflavone supplementation altered the global miRNA expression profile of colostrum-derived exosomes

Principal component analysis (PCA) demonstrated a clear separation between the CON and SIF groups along PC1, which accounted for 99.55% of the total variance, indicating that soy isoflavone supplementation markedly altered the global miRNA expression profile of colostrum-derived exosomes (Fig. 2A). Samples within each group clustered closely, suggesting high reproducibility. Pearson correlation analysis further confirmed the reliability of the sequencing data, with correlation coefficients exceeding 0.98 among samples and strong intra-group consistency (Fig. 2B). The distribution of differentially expressed miRNAs revealed that a number of miRNAs were significantly upregulated or downregulated between the SIF and CON groups under different statistical thresholds. At $P < 0.05$, 24 miRNAs were upregulated and 23 miRNAs were downregulated in the SIF group compared with the CON group (Fig. 2C). Hierarchical clustering analysis showed that these differentially expressed miRNAs clearly distinguished the CON and SIF samples, further supporting that soy isoflavone supplementation reshaped the miRNA cargo of colostrum-derived exosomes (Fig. 2D). The volcano analysis revealed a clear pattern of miRNA expression changes in the SIF group, including a number of markedly elevated and reduced miRNAs (Fig. 2E).

1. (A) Principal component analysis (PCA) of exosomal miRNA expression profiles in the CON and SIF groups.
2. (B) Pearson correlation heatmap showing the correlation coefficients among samples.
3. (C) Numbers of upregulated and downregulated miRNAs between the SIF and CON groups under different statistical thresholds.
4. (D) Hierarchical clustering heatmap of differentially expressed miRNAs in colostrum-derived exosomes.
5. (E) Volcano plot showing the distribution of differentially expressed miRNAs between the SIF and CON groups. Red dots indicate significantly upregulated miRNAs, blue dots indicate significantly downregulated miRNAs, and gray dots indicate non-significant miRNAs.

Functional characteristics of differentially expressed miRNAs

Further functional characterization of differentially expressed miRNAs revealed that the upregulated miRNAs in the SIF group were mainly associated with inflammatory regulation, epithelial homeostasis, and oxidative stress-related pathways (Table 5, Table S1). For instance, miR-181a, miR-339-3p, and miR-532-3p have been reported to participate in immune modulation and inflammatory signaling [19, 20]. MiR-200b and miR-138 are involved in epithelial differentiation and tight junction maintenance [21, 22], whereas miR-484 has been linked to mitochondrial function and oxidative stress regulation [23]. In contrast, several downregulated miRNAs in the SIF group were previously implicated in inflammatory amplification, lipid metabolism, and signaling pathway regulation, including miR-21-3p, miR-132, and members of the miR-27 family [24–26]. Additionally, reduced expression of let-7 family members (let-7d-5p and let-7f-5p) suggests potential alterations in miRNAs involved in cell differentiation and developmental processes [27]. Collectively, these findings indicate that soy isoflavone supplementation markedly altered the expression of multiple miRNAs associated with inflammation, metabolism, and epithelial function, suggesting a reshaping of the miRNA cargo in colostrum-derived exosomes.

Table 5
Top 10 upregulated and downregulated exosomal miRNAs in sow colostrum from the SIF group compared with the CON group

miRNA	Regulation	Fold change (SIF/CON)	log_2FC	*P* - value
Top 10 upregulated miRNAs				
cfa-miR-200b	Up	1.178	0.237	0.0044
ssc-miR-339-3p	Up	2.091	1.065	0.0084
PC-3p-7860_357	Up	2.528	1.338	0.0111
ssc-miR-532-3p	Up	1.830	0.872	0.0150
ocu-miR-138-5p_R + 1	Up	Inf*	Inf*	0.0150
ssc-miR-181a_R-1	Up	1.730	0.790	0.0157
ocu-miR-18a-3p_R-1	Up	1.597	0.675	0.0157
hsa-miR-4286_R + 4	Up	2.360	1.239	0.0210
ssc-mir-4332-p3_1ss18TG	Up	7.368	2.881	0.0219
ssc-miR-339_R-1	Up	2.145	1.101	0.0250
Top 10 downregulated miRNAs				
ssc-miR-27b-5p_R-1	Down	0.551	-0.860	0.0019
ocu-miR-340-3p	Down	0.652	-0.617	0.0033
ssc-let-7d-5p	Down	0.778	-0.363	0.0039
ssc-miR-23b_R-1	Down	0.620	-0.689	0.0185
ocu-miR-98-3p_1ss22CT	Down	0.706	-0.503	0.0188
ssc-miR-30e-3p_1ss22CT	Down	0.733	-0.449	0.0215
ssc-miR-135	Down	0.300	-1.738	0.0235
ssc-miR-374a-3p	Down	0.801	-0.320	0.0293
PC-3p-32970_43	Down	0.322	-1.639	0.0323
ssc-let-7f-5p	Down	0.780	-0.359	0.0328

Functional enrichment analysis of predicted target genes

Functional characterization of the predicted targets of the differentially expressed miRNAs was carried out through GO term annotation and KEGG pathway mapping (Fig. 3), and the full enrichment results are listed in Supplementary Tables S2 and S3. These tables include the enriched terms/pathways, associated target genes, enrichment ratios, and adjusted significance values. GO enrichment analysis indicated significant enrichment in terms associated with intracellular localization and transcriptional regulation, including cytoplasm, nucleus, cytosol, intracellular membrane-bounded organelle, and regulation of transcription by RNA polymerase II. Additionally, terms related to protein phosphorylation, kinase activity, and metal ion binding were significantly enriched, suggesting potential involvement of the differentially expressed miRNAs in signal transduction and kinase-mediated regulatory processes (Fig. 3A). Subsequent KEGG annotation suggested that the differentially expressed miRNA targets were preferentially associated with a range of biological pathways, notably MAPK, Ras, and Rap1 signaling, together with endocytosis, autophagy-animal, and adherents junction pathways. Enrichment was also observed in broader pathways such as metabolic pathways and pathways in cancer. Several pathways exhibited relatively high rich factor values and strong statistical significance, indicating that these miRNAs may participate in regulating key signaling cascades, cellular proliferation, and structural homeostasis (Fig. 3B). Collectively, the GO and KEGG enrichment results suggest that SIF-associated differentially expressed miRNAs are mainly

involved in transcriptional regulation, signaling pathway modulation, and cellular structural organization, providing a functional basis for subsequent mechanistic interpretation.

1. (A) Gene Ontology (GO) enrichment scatter plot of predicted target genes of differentially expressed miRNAs.
2. (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment scatter plot of predicted target genes of differentially expressed miRNAs. Dot size represents gene number, and color indicates enrichment significance.

Integrated regulatory network analysis of DE-miRNAs

To further elucidate the regulatory mechanisms of differentially expressed miRNAs, integrated miRNA-mRNA, miRNA-mRNA-GO, and ceRNA networks were constructed. In the miRNA-mRNA network, several inflammation- and signaling-related genes, including Tnf, Il1b, Ccl3, Ccr5, Tgfb1, Mapk12, Map3k8, Pak1, and Pak3, were identified as central nodes with high connectivity (Fig. 4A). These genes were targeted by multiple differentially expressed miRNAs, suggesting that they may serve as key regulatory hubs involved in signal transduction and inflammatory responses. The miRNA-mRNA-GO network further indicated that core target genes were primarily enriched in signal transduction-related functional categories, supporting the potential involvement of DE-miRNAs in regulating MAPK-associated pathways and immune-related processes (Fig. 4B). KEGG-based association analysis revealed that several DE-miRNAs potentially regulated genes such as AAAS, AARS1, AADAT, and AACS, which were enriched in aminoacyl-tRNA biosynthesis, RNA transport, and amino acid metabolism pathways (Fig. 4C). These findings suggest a possible role of miRNAs in modulating protein synthesis and metabolic homeostasis. Moreover, the constructed ceRNA network suggested that multiple lncRNAs and circRNAs may competitively bind miRNAs to regulate key genes including CFTR, DBNDD1, FKBP4, and M6PR, indicating a multi-layered post-transcriptional regulatory mechanism (Fig. 4D). Collectively, these results suggest that DE-miRNAs may participate in the regulatory response to SIF supplementation through coordinated modulation of inflammatory signaling axes, metabolic pathways, and ceRNA-mediated interactions.

1. (A) Integrated miRNA-mRNA interaction network showing potential regulatory relationships between differentially expressed miRNAs and their predicted target genes. Orange nodes represent mRNAs, and blue nodes represent miRNAs.
2. (B) miRNA-mRNA-GO network showing the associations among differentially expressed miRNAs, predicted target genes, and significantly enriched GO terms. Yellow nodes represent GO terms.
3. (C) Sankey diagram of miRNA-gene-KEGG pathway associations, illustrating the potential involvement of differentially expressed miRNAs in enriched metabolic and signaling pathways.
4. (D) ceRNA regulatory network showing potential interactions among lncRNAs/circRNAs, miRNAs, and mRNAs, indicating a multi-layered post-transcriptional regulatory mechanism.

Discussion

Soy isoflavones (SIF) are naturally occurring phytoestrogens widely present in soybean and soybean-derived feed ingredients, which are major protein sources in swine diets. Due to their structural similarity to endogenous estrogens, SIF can interact with estrogen receptors and regulate multiple physiological processes, including reproductive function, antioxidant defense, and immune responses [9]. Previous studies have demonstrated that dietary SIF supplementation can improve antioxidant status, modulate inflammatory responses, and influence metabolic regulation in pigs [8]. Therefore, SIF have attracted considerable attention as functional feed additives in sow nutrition.

In the present study, dietary supplementation with soy isoflavones significantly improved sow reproductive performance, as evidenced by increased total born and live-born piglets per litter, greater litter weight at birth and at weaning, and a reduced farrowing duration. These results suggest that SIF supplementation may enhance reproductive efficiency and piglet vitality. Similar findings have been reported in previous studies, that dietary phytoestrogen supplementation improved litter size and offspring growth performance in pigs and other livestock species [28]. The beneficial effects of SIF on reproductive performance may be partly attributed to their estrogen-like activity, which enables them to modulate reproductive-related physiological processes. Oxidative stress during late gestation and lactation is widely recognized as a major factor affecting reproductive outcomes and lactation performance in sows. Excessive oxidative stress may impair placental function, prolong parturition, and negatively influence milk production and neonatal vitality [29]. In the present study, dietary SIF supplementation significantly increased serum total antioxidant capacity and catalase activity in lactating sows, indicating an enhanced antioxidant defense system. These results

are consistent with previous reports showing that soy isoflavones can improve antioxidant status by enhancing antioxidant enzyme activities and reducing oxidative damage. The improved antioxidant capacity may help maintain physiological homeostasis during the periparturient period, thereby supporting reproductive performance and piglet growth [8]. Taken together, these findings suggest that dietary SIF supplementation may improve reproductive outcomes in sows, at least in part, through enhancing maternal antioxidant capacity. These physiological improvements may also be associated with molecular regulatory mechanisms mediated by bioactive components in colostrum, including exosomal microRNAs.

Milk-derived exosomes have recently attracted considerable attention as important mediators of maternal–offspring communication due to their ability to transport bioactive molecules [30]. In the present study, exosomes were successfully isolated from sow colostrum and exhibited typical morphological and physicochemical characteristics. Transmission electron microscopy showed the classical cup-shaped or spherical vesicles, while nanoparticle tracking analysis indicated that most particles ranged from 30 to 150 nm with an average diameter of approximately 83 nm. These features are consistent with the commonly reported size and morphology of exosomes derived from milk and other biological fluids [31]. In addition, the high positivity rate detected by flow cytometry further confirmed the purity and successful isolation of colostrum-derived exosomes. Small RNA sequencing revealed that the majority of miRNAs ranged from 18 to 26 nucleotides in length, with a dominant peak at 22–23 nt. This size distribution is consistent with the typical length of mature miRNAs generated through the canonical Dicer processing pathway and has been widely reported in previous miRNA sequencing studies [32]. The observed length profile therefore indicates the reliability and high quality of the small RNA libraries constructed in this study. Moreover, the comparable exosome size distribution between the CON and SIF groups suggests that dietary soy isoflavone supplementation did not alter the physical characteristics of colostrum exosomes but may instead influence their molecular cargo, particularly miRNA composition. These results provide a solid foundation for further analysis of differential miRNA expression and the exploration of potential regulatory mechanisms mediated by colostrum-derived exosomal miRNAs.

MicroRNAs carried by milk-derived exosomes are considered key regulators of maternal–offspring communication and may influence early-life physiological development [33]. In the present study, principal component analysis revealed a clear separation between the CON and SIF groups, indicating that dietary soy isoflavone supplementation markedly altered the global miRNA expression profile of colostrum-derived exosomes. The high correlation coefficients among samples and the consistent clustering within each group further confirmed the reliability of the sequencing data and the reproducibility of the experimental results. Moreover, differential expression analysis identified a number of miRNAs that were significantly upregulated or downregulated in response to SIF supplementation, suggesting that maternal dietary phytoestrogens can reshape the molecular cargo of milk-derived exosomes. Emerging evidence indicates that maternal nutrition can influence the composition of milk-derived exosomal miRNAs, thereby modulating signaling pathways involved in immunity, metabolism, and intestinal development in offspring [34]. Previous studies have shown that dietary bioactive compounds, including plant-derived polyphenols and phytoestrogens, can regulate miRNA expression through epigenetic and transcriptional mechanisms [35]. Given the estrogen-like activity and antioxidant properties of soy isoflavones, it is plausible that SIF supplementation may affect miRNA biogenesis or secretion in mammary epithelial cells, leading to altered miRNA profiles in colostrum exosomes [36]. These miRNAs may subsequently participate in maternal-offspring molecular signaling and influence neonatal physiological processes. To further explore the potential biological functions of these differentially expressed miRNAs, target gene prediction and functional enrichment analyses were performed.

The functional characterization of differentially expressed miRNAs in this study suggested that dietary soy isoflavone supplementation markedly influenced regulatory networks associated with inflammation, epithelial homeostasis, and oxidative stress. Several miRNAs that were upregulated in the SIF group, including miR-181a, miR-339-3p, and miR-532-3p, have previously been reported to participate in immune regulation and inflammatory signaling pathways. For example, miR-181a has been shown to modulate immune cell activation and cytokine production, while miR-532-3p has been implicated in the regulation of inflammatory responses and cellular stress signaling [37]. The increased expression of these miRNAs may therefore reflect an enhanced regulatory capacity for maintaining immune and inflammatory balance. In addition, miRNAs associated with epithelial integrity were also affected by SIF supplementation. MiR-200b and miR-138 have been reported to regulate epithelial differentiation and maintain tight junction stability, which are critical for intestinal barrier function [38]. Meanwhile, miR-484 has been linked to mitochondrial activity and oxidative stress regulation, suggesting a potential role in cellular energy metabolism and antioxidant defense [39]. These observations are consistent with the known biological functions of soy isoflavones, which possess antioxidant and anti-inflammatory properties. Conversely, several miRNAs that were downregulated in the SIF group, such as miR-21-3p, miR-132, and members of the miR-27 family, have been previously associated with inflammatory amplification, lipid metabolism, and signal

transduction pathways [40]. The reduced expression of these miRNAs, together with decreased levels of let-7 family members, may indicate a shift in regulatory networks involved in cell differentiation and metabolic regulation. Collectively, these results suggest that maternal dietary SIF supplementation reshapes the functional landscape of colostrum-derived exosomal miRNAs, potentially influencing inflammatory responses, epithelial development, and metabolic processes in offspring.

To further elucidate the potential biological roles of the differentially expressed miRNAs, GO and KEGG enrichment analyses were performed based on their predicted target genes. The GO analysis revealed significant enrichment in terms associated with intracellular localization and transcriptional regulation, including cytoplasm, nucleus, and regulation of transcription by RNA polymerase II. These findings suggest that the differentially expressed miRNAs may participate in post-transcriptional regulatory processes and intracellular signaling networks. In addition, enrichment of terms related to protein phosphorylation, kinase activity, and metal ion binding indicates the potential involvement of these miRNAs in kinase-mediated signal transduction and regulatory cascades. KEGG pathway analysis further showed that the predicted target genes were significantly enriched in several key signaling pathways, including the MAPK, Ras, and Rap1 signaling pathways, as well as biological processes such as endocytosis, autophagy, and adherens junction formation. These pathways are known to play essential roles in regulating cellular proliferation, immune responses, oxidative stress adaptation, and epithelial barrier integrity. Previous studies have also demonstrated that soy isoflavones can modulate MAPK and related signaling pathways, contributing to their antioxidant and anti-inflammatory effects [41]. Therefore, the enrichment of these pathways in the present study may partly explain the physiological improvements observed in SIF-supplemented sows, such as enhanced antioxidant capacity and improved reproductive performance. Collectively, these results suggest that maternal dietary SIF supplementation may regulate multiple intracellular signaling pathways through exosomal miRNA-mediated mechanisms. Such regulatory effects may contribute to maintaining cellular homeostasis and facilitating maternal-offspring molecular communication during early-life development.

To further elucidate the molecular basis underlying SIF-associated miRNA alterations, integrated miRNA-mRNA, miRNA-mRNA-GO, and ceRNA networks were constructed. The miRNA-mRNA network highlighted several inflammation- and signaling-related genes (e.g., Tnf, Il1b, Ccl3, Ccr5, Tgfb1, Mapk12, Map3k8, Pak1, and Pak3) as highly connected nodes. These genes are well-recognized regulators of inflammatory responses and kinase-driven signaling cascades, particularly within MAPK-related pathways. The observation that multiple differentially expressed miRNAs converged on these hub genes suggests a coordinated post-transcriptional regulatory pattern, which may contribute to fine-tuning inflammatory signaling and cellular stress responses [42]. Consistently, the miRNA–mRNA–GO network indicated that core target genes were primarily enriched in signal transduction–related categories, supporting the potential involvement of DE-miRNAs in MAPK-associated regulation and immune-related processes. Given that soy isoflavones possess antioxidant and estrogen-like activities and have been reported to modulate inflammatory signaling, these network-level findings provide a plausible molecular link between maternal SIF supplementation and the improved physiological outcomes observed in sows (e.g., enhanced antioxidant capacity and reproductive performance) [43, 44]. In addition to inflammatory signaling, KEGG-based association analysis suggested that several DE-miRNAs may regulate genes involved in aminoacyl-tRNA biosynthesis, RNA transport, and amino acid metabolism, implying potential effects on protein synthesis and metabolic homeostasis during lactation [45]. Moreover, the ceRNA network indicated that multiple lncRNAs and circRNAs could competitively bind miRNAs to regulate genes such as CFTR, DBNDD1, FKBP4, and M6PR, highlighting a multilayered post-transcriptional regulatory architecture [46]. Collectively, these results suggest that SIF supplementation may reshape colostrum exosomal miRNA cargo and its upstream ceRNA interactions, thereby coordinately modulating inflammatory signaling axes and metabolic pathways within the maternal–offspring regulatory framework.

Conclusion

Dietary soy isoflavone supplementation significantly improved sow reproductive performance, as indicated by increased total born and live-born piglets per litter, higher litter weight at birth and at weaning, and a shortened farrowing duration. Soy isoflavones also enhanced maternal antioxidant capacity on day 21 of lactation, reflected by elevated serum total antioxidant capacity and catalase activity. Colostrum-derived exosomes were successfully isolated and characterized, and small RNA sequencing showed a typical miRNA length distribution (18–26 nt) with a predominant peak at 22–23 nt, supporting good library quality. Comparative profiling further demonstrated that soy isoflavones markedly reshaped the miRNA cargo of colostrum-derived exosomes. Functional enrichment and integrated network analyses of predicted targets suggested that SIF-associated miRNAs are mainly linked to transcriptional regulation, kinase-mediated signaling, inflammatory signaling axes, and metabolic pathways, potentially involving

multilayered post-transcriptional regulation. Further studies with experimental validation are warranted to confirm key miRNA–target interactions and clarify their functional relevance to offspring development.

Abbreviations

Abbreviations used in this manuscript are listed in **Supplementary Table 4**.

Declarations

Authors' contributions

JH and JL conceived and designed the experiments. QD performed animal trial and wrote the manuscript. XL, KX, ZP, YL, and HY performed biochemical analysis. XM and HY gave constructive comments for the results and discussion of the manuscript. All authors have read and approved the final manuscript.

Funding

This study was supported by the National Key R&D Program of China (2023YFD1301200) and the Porcine Innovation Team of Sichuan Province (SCCXTD-2024-8).

Availability of data and materials

The miRNA-seq data generated in this study have been deposited in the NCBI Gene Expression Omnibus (GEO) under accession number GSE327773.

Declarations

All experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University (No. 20181105). The experiment was conducted at a commercial pig farm owned by Luoshi Animal Husbandry Co., Ltd. in Wangcang County, Guangyuan City, Sichuan Province, China. Written informed consent was obtained from the farm owner/management before the study was conducted.

Acknowledgements

We extend our sincere gratitude to Tingting Jiang at LC Bio-Technology Co., Ltd. for her expert guidance and outstanding technical support in bioinformatics analyses.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no conflicts of interest.

References

1. Ji Y, Wu Z, Dai Z, Wang X, Li J, Wang B, Wu G. Fetal and neonatal programming of postnatal growth and feed efficiency in swine. J Anim Sci Biotechnol. 2017;8:42. 10.1186/s40104-017-0173-5.
2. Maciag SS, Bellaver FV, Bombassaro G, Haach V, Morés MAZ, Baron LF, et al. On the influence of the source of porcine colostrum in the development of early immune ontogeny in piglets. Sci Rep. 2022;12:15630. 10.1038/s41598-022-20082-1.
3. Smith BN, Dilger RN. Immunomodulatory potential of dietary soybean-derived isoflavones and saponins in pigs. J Anim Sci. 2018;96:1288–304. 10.1093/jas/sky036.
4. Ariyani W, Koibuchi N. The effect of soy isoflavones in brain development: the emerging role of multiple signaling pathways and future perspectives. Endocr J. 2024;71:317–33. 10.1507/endocrj.EJ23-0314.

5. Li H, Liu Z, Lyu H, Gu X, Song Z, He X, Fan Z. Effects of dietary inulin during late gestation on sow physiology, farrowing duration and piglet performance. Anim Reprod Sci. 2020;219:106531. 10.1016/j.anireprosci.2020.106531.
6. Dumniem N, Suwimonteerabutr J, Tummaruk P. Colostrum and milk production in sows housed in free-farrowing versus crated systems under tropical conditions: Associations with sow metabolic state, oxidative stress, and piglet survival. Theriogenology. 2025;247:117572. 10.1016/j.theriogenology.2025.117572.
7. Huang L, Ma X-Y, Jiang Z-Y, Hu Y-J, Zheng C-T, Yang X-F, et al. Effects of soybean isoflavone on intestinal antioxidant capacity and cytokines in young piglets fed oxidized fish oil. J Zhejiang Univ Sci B. 2016;17:965–74. 10.1631/jzus.B1600078.
8. Li YP, Jiang XR, Wei ZX, Cai L, Yin JD, Li XL. Effects of soybean isoflavones on the growth performance, intestinal morphology and antioxidative properties in pigs. Animal. 2020;14:2262–70. 10.1017/S1751731120001123.
9. Li Y, Jiang X, Cai L, Zhang Y, Ding H, Yin J, Li X. Effects of daidzein on antioxidant capacity in weaned pigs and IPEC-J2 cells. Anim Nutr. 2022;11:48–59. 10.1016/j.aninu.2022.06.014.
10. Jin J, Liu S, Zhou Q, Fang Z, Lin Y, Xu S, et al. Cinnamaldehyde supplementation in sows and their offspring: effects on colostrum and milk composition, performance, redox status and intestinal health. J Anim Sci Biotechnol. 2025;16:79. 10.1186/s40104-025-01212-x.
11. Chen R, Yang H, Dai J, Zhang M, Lu G, Zhang M, et al. The biological functions of maternal-derived extracellular vesicles during pregnancy and lactation and its impact on offspring health. Clin Nutr. 2023;42:493–504. 10.1016/j.clnu.2023.02.007.
12. Samuel M, Chisanga D, Liem M, Keerthikumar S, Anand S, Ang C-S, et al. Bovine milk-derived exosomes from colostrum are enriched with proteins implicated in immune response and growth. Sci Rep. 2017;7:5933. 10.1038/s41598-017-06288-8.
13. Liao Y, Du X, Li J, Lönnerdal B. Human milk exosomes and their microRNAs survive digestion in vitro and are taken up by human intestinal cells. Mol Nutr Food Res. 2017. 10.1002/mnfr.201700082.
14. Chen T, Xie M-Y, Sun J-J, Ye R-S, Cheng X, Sun R-P, et al. Porcine milk-derived exosomes promote proliferation of intestinal epithelial cells. Sci Rep. 2016;6:33862. 10.1038/srep33862.
15. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116:281–97. 10.1016/s0092-8674(04)00045-5.
16. Niazi SK, Magoola M. MicroRNA Nobel Prize: Timely Recognition and High Anticipation of Future Products-A Prospective Analysis. Int J Mol Sci. 2024. 10.3390/ijms252312883.
17. Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. J Biol Chem. 2010;285:17442–52. 10.1074/jbc.M110.107821.
18. Cornelis de Lange, New NRC. (2012) Nutrient Requirements of Swine. In:; 2014.
19. Chen F, Xing Y, Chen Z, Chen X, Li J, Gong S, et al. Competitive adsorption of microRNA-532-3p by circular RNA SOD2 activates Thioredoxin Interacting Protein/NLR family pyrin domain containing 3 pathway and promotes pyroptosis of non-alcoholic fatty hepatocytes. Eur J Med Res. 2024;29:250. 10.1186/s40001-024-01817-4.
20. Zhang M, Zhang Q, Hu Y, Xu L, Jiang Y, Zhang C, et al. miR-181a increases FoxO1 acetylation and promotes granulosa cell apoptosis via SIRT1 downregulation. Cell Death Dis. 2017;8:e3088. 10.1038/cddis.2017.467.
21. Li Y, Ling P, Li Y, Wang Y, Li G, Qiu C, et al. miR-138-5p ameliorates intestinal barrier disruption caused by acute superior mesenteric vein thrombosis injury by inhibiting the NLRP3/HMGB1 axis. PeerJ. 2024;12:e16692. 10.7717/peerj.16692.
22. Shen Y, Zhou M, Yan J, Gong Z, Xiao Y, Zhang C, et al. miR-200b inhibits TNF-α-induced IL-8 secretion and tight junction disruption of intestinal epithelial cells in vitro. Am J Physiol Gastrointest Liver Physiol. 2017;312:G123–32. 10.1152/ajpgi.00316.2016.
23. Li H, Wang X, Mu H, Mei Q, Liu Y, Min Z, et al. Mir-484 contributes to diminished ovarian reserve by regulating granulosa cell function via YAP1-mediated mitochondrial function and apoptosis. Int J Biol Sci. 2022;18:1008–21. 10.7150/ijbs.68028.
24. Ru L, Wang X-M, Niu J-Q. The miR-23-27-24 cluster: an emerging target in NAFLD pathogenesis. Acta Pharmacol Sin. 2022;43:1167–79. 10.1038/s41401-021-00819-w.
25. Mishra N, Friedson L, Hanin G, Bekenstein U, Volovich M, Bennett ER, et al. Antisense miR-132 blockade via the AChE-R splice variant mitigates cortical inflammation. Sci Rep. 2017;7:42755. 10.1038/srep42755.
26. Wang W, Qiao S, Kong X, Zhang G, Cai Z. The role of exosomes in immunopathology and potential therapeutic implications. Cell Mol Immunol. 2025;22:975–95. 10.1038/s41423-025-01323-5.

27. Wells AC, Daniels KA, Angelou CC, Fagerberg E, Burnside AS, Markstein M, et al. Modulation of let-7 miRNAs controls the differentiation of effector CD8 T cells. Elife. 2017. 10.7554/eLife.26398.
28. Luo W, Yin X, Yao J, Cheng J, Zhang J, Xu W, et al. Fermented Soybean Meal Affects the Reproductive Performance and Oxidative Status of Sows, and the Growth of Piglets. Anim (Basel). 2021. 10.3390/ani11030597.
29. Yuan X, Zhang X, Wu Y, Che D, Ye H, Pi Y, et al. Maternal Amino Acid Mixtures Supplementation during Late Gestation and Lactation Improved Growth Performance of Piglets through Improving Colostrum Composition and Antioxidant Capacity. Antioxid (Basel). 2022. 10.3390/antiox11112144.
30. Lu D, Liu Y, Kang L, Zhang X, Hu J, Ye H, et al. Maternal fiber-rich diet promotes early-life intestinal development in offspring through milk-derived extracellular vesicles carrying miR-146a-5p. J Nanobiotechnol. 2024;22:65. 10.1186/s12951-024-02344-4.
31. Thompson AG, Gray E, Heman-Ackah SM, Mäger I, Talbot K, Andaloussi SE, et al. Extracellular vesicles in neurodegenerative disease - pathogenesis to biomarkers. Nat Rev Neurol. 2016;12:346–57. 10.1038/nrneurol.2016.68.
32. Li X, Zhang Z, Wang X, Lu L, Zhang Z, Zhang G, et al. In Vitro Analysis of LPS-Induced miRNA Differences in Bovine Endometrial Cells and Study of Related Pathways. Anim (Basel). 2024. 10.3390/ani14233367.
33. Lowry DE, Paul HA, Reimer RA. Impact of maternal obesity and prebiotic supplementation on select maternal milk microRNA levels and correlation with offspring outcomes. Br J Nutr. 2022;127:335–43. 10.1017/S0007114521001197.
34. Martz J, Hammer B, Langen TJ, Berkowitz B, Berkowitz B, Storm JA et al. Investigating milk-derived extracellular vesicles as mediators of maternal stress and environmental intervention. bioRxiv. 2025. 10.1101/2025.05.30.656911
35. Villagrán-Andrade KM, Núñez-Carro C, Blanco FJ, de Andrés MC. Nutritional Epigenomics: Bioactive Dietary Compounds in the Epigenetic Regulation of Osteoarthritis. Pharmaceuticals (Basel). 2024. 10.3390/ph17091148.
36. Ma X, Cui Y, Tian Z, Yu M. Effect of Soybean Isoflavones on Proliferation and Related Gene Expression of Sow Mammary Gland Cells In Vitro. Anim (Basel). 2022. 10.3390/ani12233241.
37. Tsitsiou E, Lindsay MA. microRNAs and the immune response. Curr Opin Pharmacol. 2009;9:514–20. 10.1016/j.coph.2009.05.003.
38. Sun D, Cao H, Yang L, Lin L, Hou B, Zheng W, et al. MiR-200b in heme oxygenase-1-modified bone marrow mesenchymal stem cell-derived exosomes alleviates inflammatory injury of intestinal epithelial cells by targeting high mobility group box 3. Cell Death Dis. 2020;11:480. 10.1038/s41419-020-2685-8.
39. Atiaa AG, Abd E-, Ellakwa DE-S. Crosstalk between microRNA and oxidative stress in ovarian cancer: diagnosis, pathogenesis and therapeutic resistance. Med Oncol. 2025;43:104. 10.1007/s12032-025-03024-5.
40. Fatima Z, Itrat N, Israr B, Ahmad AMR. Therapeutic Efficacy of Soy-Derived Bioactives: A Systematic Review of Nutritional Potency, Bioactive Therapeutics, and Clinical Biomarker Modulation. Foods. 2025. 10.3390/foods14193447
41. Li K, Hu W, Yang Y, Wen H, Li W, Wang B. Anti-inflammation of hydrogenated isoflavones in LPS-stimulated RAW264.7 cells via inhibition of NF-κB and MAPK signaling pathways. Mol Immunol. 2023;153:126–34. 10.1016/j.molimm.2022.11.019.
42. Lu L, McCurdy S, Huang S, Zhu X, Peplowska K, Tiirikainen M, et al. Time Series miRNA-mRNA integrated analysis reveals critical miRNAs and targets in macrophage polarization. Sci Rep. 2016;6:37446. 10.1038/srep37446.
43. Wang W, Zhuang Q, Ji K, Wen B, Lin P, Zhao Y, et al. Identification of miRNA, lncRNA and mRNA-associated ceRNA networks and potential biomarker for MELAS with mitochondrial DNA A3243G mutation. Sci Rep. 2017;7:41639. 10.1038/srep41639.
44. Zhang X, Huang F, Yang D, Peng T, Lu G. Identification of miRNA-mRNA Crosstalk in Respiratory Syncytial Virus- (RSV-) Associated Pediatric Pneumonia through Integrated miRNAome and Transcriptome Analysis. Mediators Inflamm. 2020;2020:8919534. 10.1155/2020/8919534.
45. Liu S, Li X, Xie Q, Zhang S, Liang X, Li S, Zhang P. Identification of a lncRNA/circRNA-miRNA-mRNA network in Nasopharyngeal Carcinoma by deep sequencing and bioinformatics analysis. J Cancer. 2024;15:1916–28. 10.7150/jca.91546.
46. Du K, Bai X, Chen L, Shi Y, Wang H-D, Cai M-C, et al. Integrated analysis of microRNAs, circular RNAs, long non-coding RNAs, and mRNAs revealed competing endogenous RNA networks involved in brown adipose tissue whitening in rabbits. BMC Genomics. 2022;23:779. 10.1186/s12864-022-09025-2.

Figures

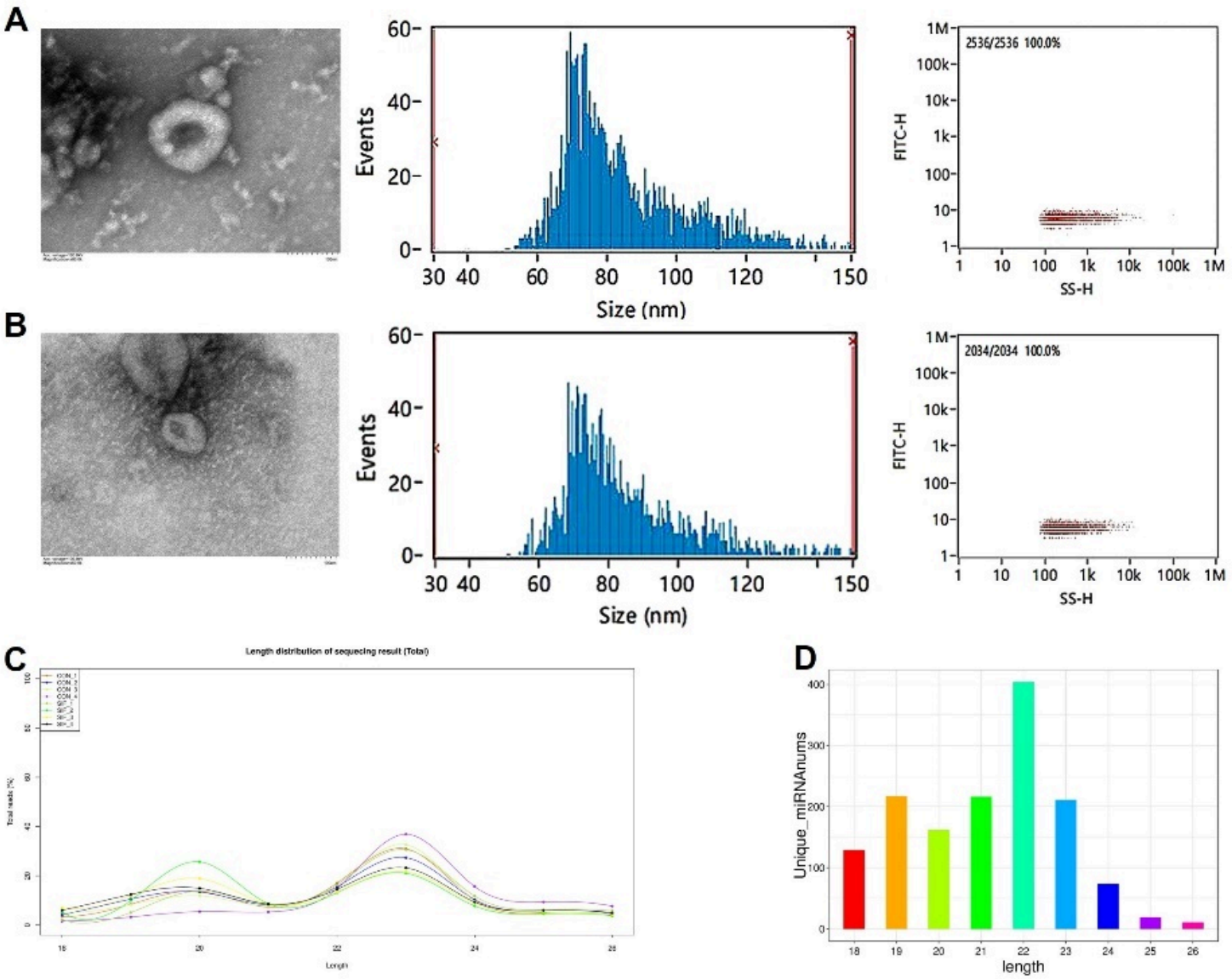


Figure 1

Characterization of colostrum-derived exosomes and small RNA length distribution.

(A–B) Transmission electron microscopy (TEM) images of exosomes isolated from sow colostrum in the CON and SIF groups, showing typical cup-shaped or spherical vesicular morphology. Nanoparticle tracking analysis (NTA) showed that the particle sizes were mainly distributed within the range of 30–150 nm. Flow cytometry analysis indicated a high proportion of positive exosomal particles in both groups.

(C) Length distribution of small RNA sequencing reads across all samples. The majority of small RNAs were distributed between 18 and 26 nt.

(D) Distribution of unique miRNA counts according to sequence length, with a predominant peak at 22–23 nt, consistent with the typical length of mature miRNAs.

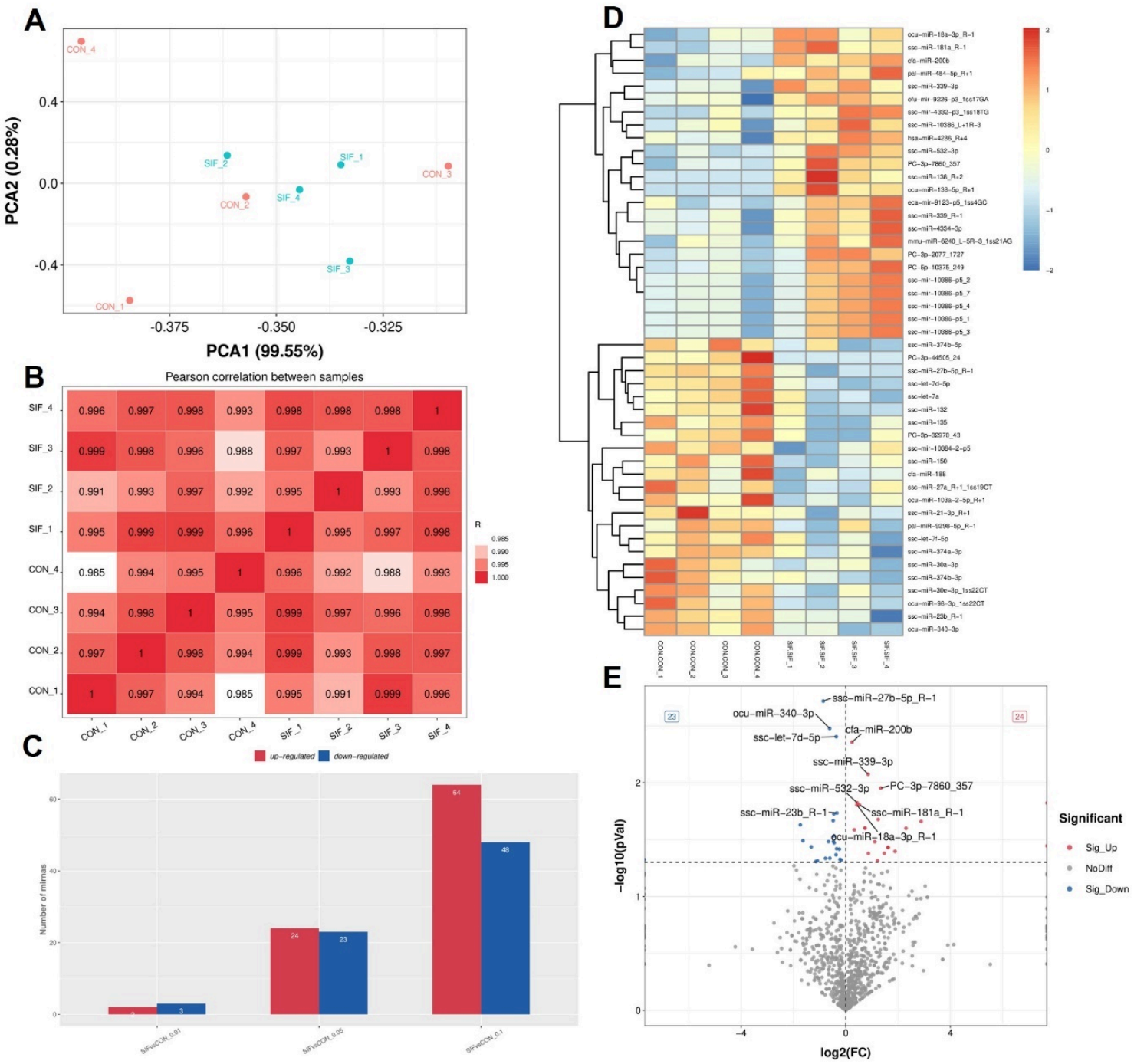


Figure 2

Soy isoflavone supplementation altered the global miRNA expression profile of colostrum-derived exosomes.

(A) Principal component analysis (PCA) of exosomal miRNA expression profiles in the CON and SIF groups.

(B) Pearson correlation heatmap showing the correlation coefficients among samples.

(C) Numbers of upregulated and downregulated miRNAs between the SIF and CON groups under different statistical thresholds.

(D) Hierarchical clustering heatmap of differentially expressed miRNAs in colostrum-derived exosomes.

(E) Volcano plot showing the distribution of differentially expressed miRNAs between the SIF and CON groups. Red dots indicate significantly upregulated miRNAs, blue dots indicate significantly downregulated miRNAs, and gray dots indicate non-significant miRNAs.

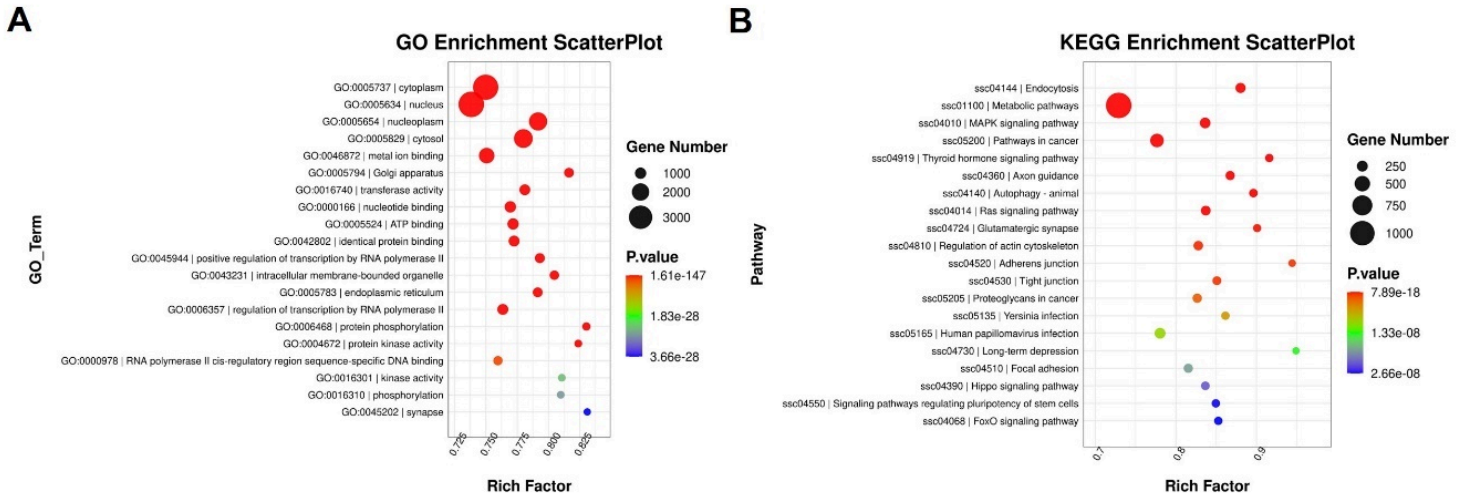


Figure 3

GO and KEGG enrichment analyses of predicted target genes of differentially expressed miRNAs.

(A) Gene Ontology (GO) enrichment scatter plot of predicted target genes of differentially expressed miRNAs.

(B) Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment scatter plot of predicted target genes of differentially expressed miRNAs. Dot size represents gene number, and color indicates enrichment significance.

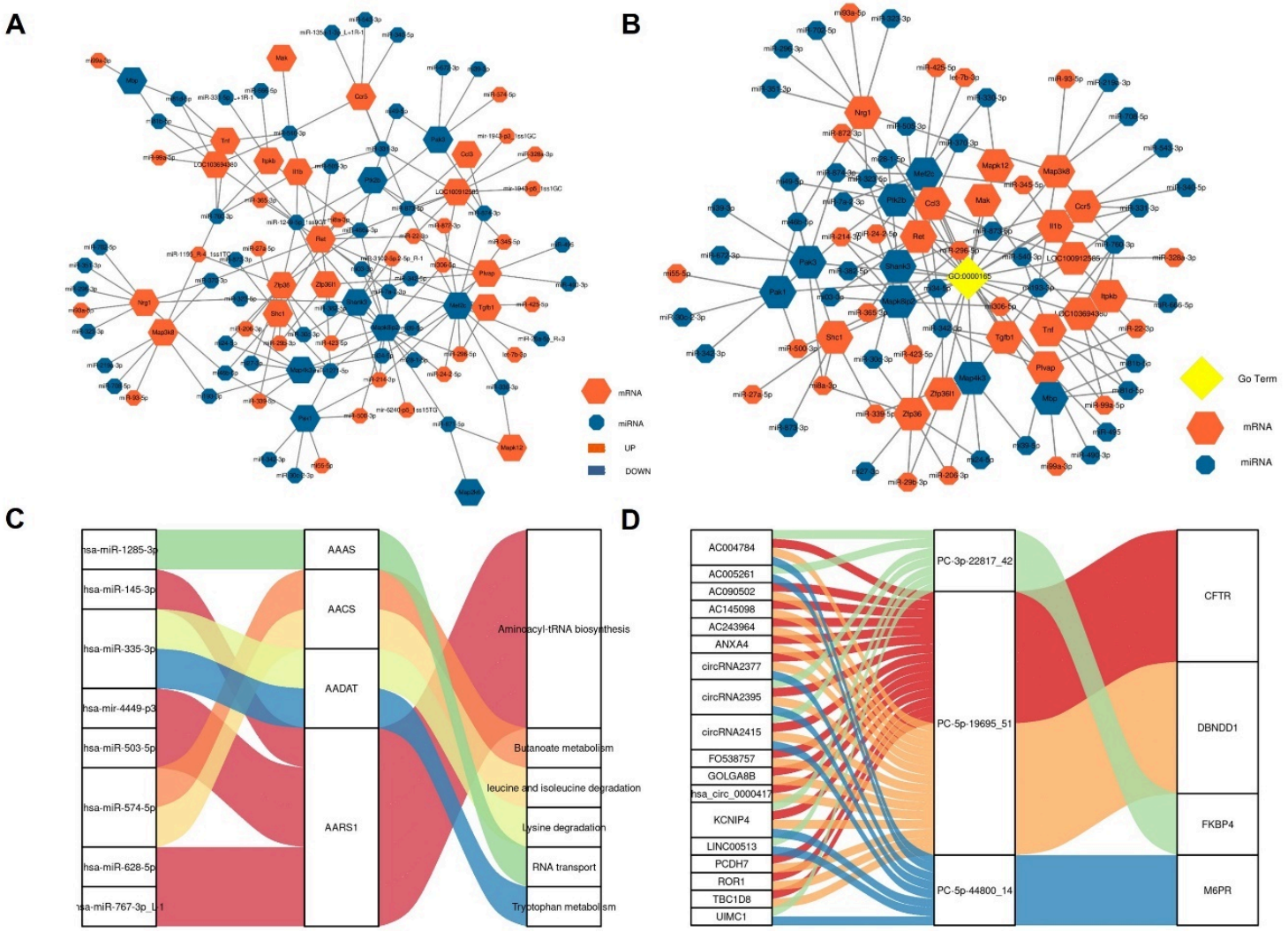


Figure 4

Integrated regulatory network analysis of differentially expressed miRNAs.

(A) Integrated miRNA-mRNA interaction network showing potential regulatory relationships between differentially expressed miRNAs and their predicted target genes. Orange nodes represent mRNAs, and blue nodes represent miRNAs.

(B) miRNA-mRNA-GO network showing the associations among differentially expressed miRNAs, predicted target genes, and significantly enriched GO terms. Yellow nodes represent GO terms.

(C) Sankey diagram of miRNA-gene-KEGG pathway associations, illustrating the potential involvement of differentially expressed miRNAs in enriched metabolic and signaling pathways.

(D) ceRNA regulatory network showing potential interactions among lncRNAs/circRNAs, miRNAs, and mRNAs, indicating a multi-layered post-transcriptional regulatory mechanism.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.xlsx
- SupplementaryTable2.xlsx
- SupplementaryTable3.xlsx
- SupplementaryTable4.docx