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Milk as MiR-acle Food: microRNA profiling of bovine milk exosomes

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CENTRE OF RESEARCH EXCELLENCE

Background:

- Milk is a complex fluid that provides nutrition, aids postnatal development, and contributes to our overall health as we age.
- MicroRNAs (miRNAs), a class of small RNAs which regulate gene expression, are selectively packaged within milk exosomes in milk which are resistant to degradation^{1, 2}.
- Bovine miRNAs share sequence similarities with human miRNAs, indicating evolutionary conservation, and putatively, conservation of function³. It is therefore possible that *bovine milk-derived miRNAs may regulate the expression of human genes*.
- Accurately profiling bovine miRNA (e.g., using direct digital quantification) is an important step in clarifying what biological role these miRNA may have in humans.

Research Design:

Exosome preparation:

Precipitation/centrifugation method (Invitrogen).

RNA extraction:

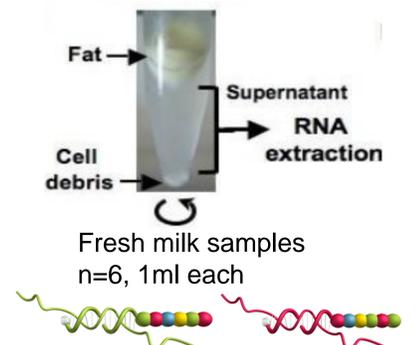
Combined phenol and column-based RNA isolation method (Invitrogen).

miRNA profiling:

NanoString (NS) nCounter[®] Human miRNA assay.

Expression and targeted pathway analyses:

nSolver[®] software (v3.0) for data normalisation, TarBase v7.0 for experimentally supported miRNA:mRNA interactions, and DIANA miRPath v3.0 for pathway analyses (KEGG and GO).



Objectives:

The objectives of the project were to:

1. Profile exosomal miRNA in bovine milk using a direct digital counting method (nCounter[®] technology) to assess if the human miRNA codeset can accurately identify bovine miRNA.
2. Assess if this technology provides *at least* similar data to milk-derived miRNA sequencing studies.
3. Explore biological pathways associated with the most abundant bovine miRNAs using *in silico* analyses.

Future work:

- Characterise miRNA abundance in pasteurised and ultra-high temperature-treated milks to assess miRNA stability after processing. This will enable a better understanding of what miRNA-mediated functionality may remain in consumer-focused products.
- Identify the roles of selected milk-derived miRNAs on the gene expression networks and molecular pathways involved in gastrointestinal function. These will be determined by further *in silico* analyses and validated with appropriate *in vitro* models.

Results:

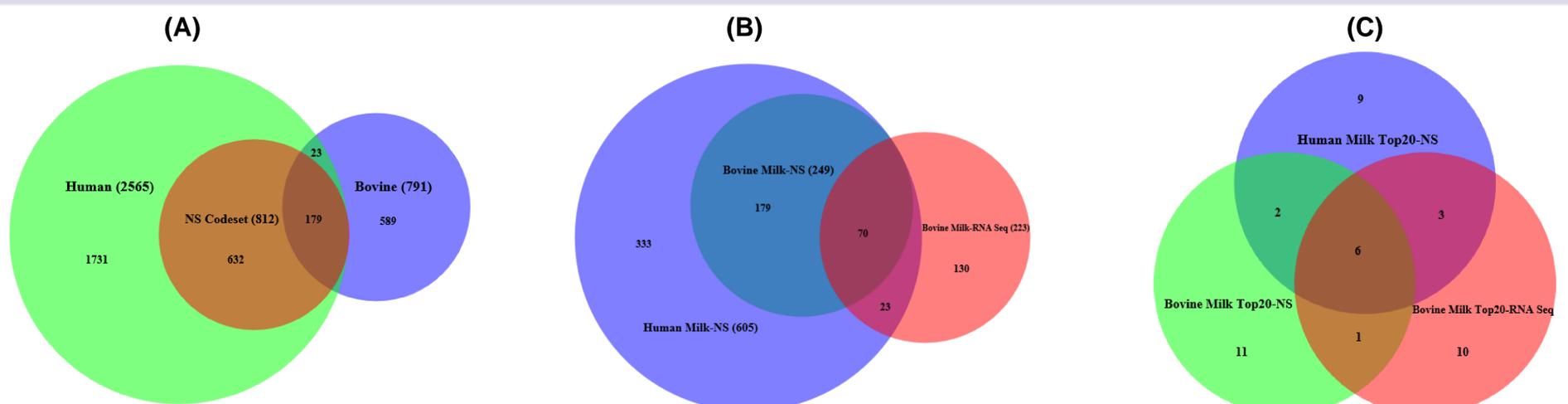


Figure 1: Venn diagrams showing the number of (A) known miRNAs in human, cow and NS assay codeset; (B) miRNAs identified in human milk exosomes by NS and in bovine milk exosomes by NS and RNA Seq⁴; (C) top 20 expressed miRNAs in human milk exosomes by NS and in bovine milk exosomes by NS and RNA Seq⁴

Table 1: Top ten highly expressed miRNAs found in bovine milk exosomes using NS nCounter[®] human miRNA assay and their identified pathways

Top 10 miRNAs in milk exosomes	Mature miRNA sequence homology	Numbers of predicted mRNA target genes	Examples of significantly enriched metabolic and cellular pathways of the identified target genes (KEGG pathways)
hsa-miR-148a-3p	100% with bta-miR-148a	1445	MicroRNAs in cancer; Proteoglycans in cancer; Viral carcinogenesis; Cell cycle;
hsa-let-7a-5p	100% with bta-let-7a-5p	2362	Pathways in cancer; Ubiquitin mediated proteolysis; Thyroid hormone signalling pathway;
hsa-let-7b-5p	100% with bta-miR-3596	2427	TGF-beta signalling pathway; Colorectal cancer; Endocytosis;
hsa-miR-4454+hsa-miR-7975	No bovine homolog was found in miRBase	88	Neurotrophin signalling pathway; Non-small cell lung cancer; p53 signalling pathway;
hsa-miR-122-5p	100% with bta-miR-122	1565	AMPK signalling pathway; Transcriptional misregulation in cancer;
hsa-miR-23a-3p	100% with bta-miR-23a	1475	Prostate cancer; Insulin signalling pathway; Lysine degradation; Fatty acid biosynthesis;
hsa-miR-200b-3p	100% with bta-miR-200b	728	Oestrogen signalling pathway; Bacterial invasion of epithelial cells; mTOR signalling pathway;
hsa-miR-200c-3p	100% with bta-miR-200c	841	Gap junction; Apoptosis; Regulation of actin cytoskeleton;
hsa-miR-141-3p	100% with bta-miR-141	902	Adipocytokine signalling pathway; MAPK signalling pathway; TNF signalling pathway.
hsa-miR-23a-3p	100% with bta-miR-23a	1475	

References:

1. Bartel DP (2009). *Cell* 136(2):215–33.
2. Izumi H, et al. (2012). *J Dairy Sci* 95(9):4831-4.
3. Esteller M (2011). *Nature Reviews Genetics* 12(12):861-74.
4. Li R, et al (2016). *PLoS One* 11(4): 806.

Acknowledgements:

- Xuejing (Ivy) Men is funded by a joint Gravida/Riddet PhD Scholarship and is based at AgResearch Limited, Grasslands Research Centre in Palmerston North, New Zealand.