microRNA Expression Profiling in Bovine Ovulation and Early Corpus Luteum Formation

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Background and objectives

Although microRNAs (miRNAs) are known to play a role in ovarian function, their specific involvement in follicle ovulation and corpus luteum (CL) formation remains unclear. Therefore, this study aimed to characterize the miRNA expression profiles of the bovine ovulatory follicle and early CL, and to identify miRNAs with potential functional significance. To achieve this, data from two independent studies were combined and analyzed.

Experimental Design

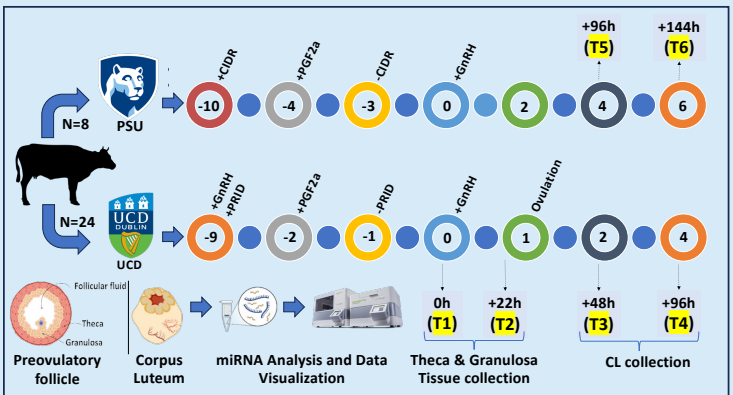

Figure 1: Oestrous synchronization protocols employed to collect follicular and luteal tissues in University College Dublin (**UCD**) and Penn State University (**PSU**). Numbers inside the coloured circles represent days relative to GnRH. Tissues were recovered at six timepoints (T): (**T1**) at time of GnRH administration (*n = 6*); (**T2**) 22 h post GnRH (*n = 6*); (**T3**) 48 h post GnRH (*n = 6*); (**T4** & **T5**) 96 h post GnRH (*n = 10*); (**T6**) 144 h post GnRH (*n = 4*). Ovaries were collected and returned to the laboratory on the ice where follicular or luteal tissue was isolated for analysis.

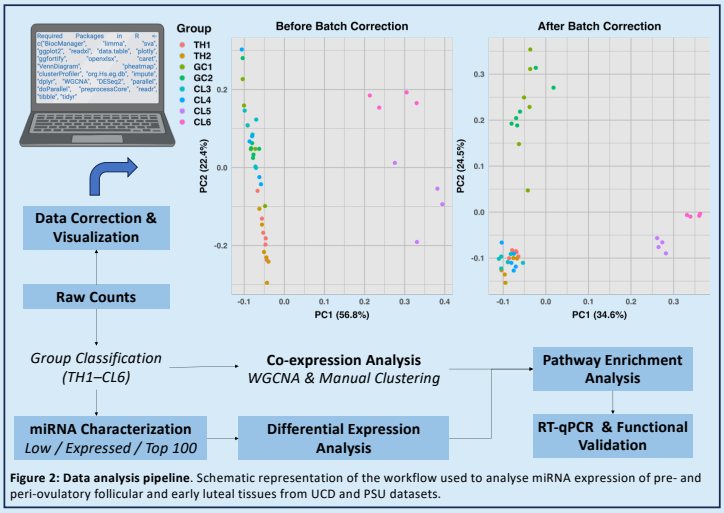

Figure 2: Data analysis pipeline. Schematic representation of the workflow used to analyse miRNA expression of pre- and peri-ovulatory follicular and early luteal tissues from UCD and PSU datasets.

Results: miRNA dynamics across ovulation and CL formation

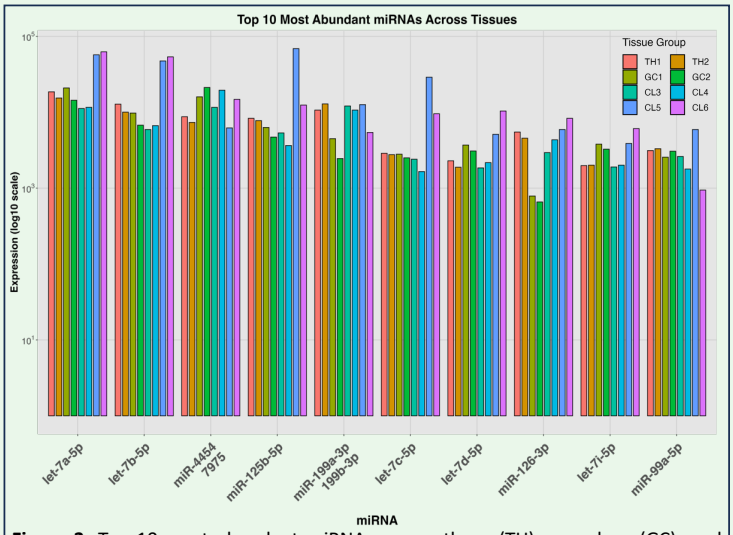

Figure 3: Top 10 most abundant miRNAs across theca (TH), granulosa (GC), and corpus luteum (CL) tissues (T1–T6). Conserved miRNAs such as the **let-7 family**, **miR-125b-5p**, and **miR-199a-3p** dominate expression, reflecting broad roles in cell differentiation and homeostasis.

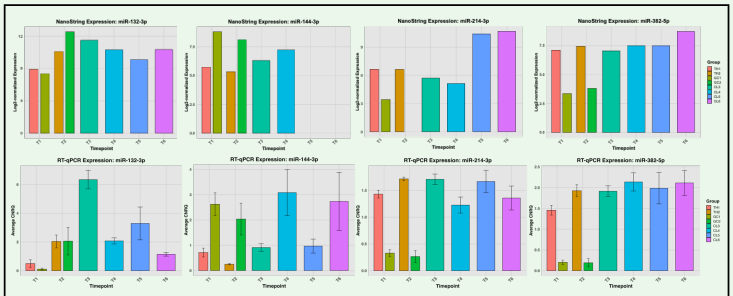

Figure 6: Validation of four candidate miRNAs (**miR-132-3p**, **miR-144-3p**, **miR-214-3p**, **miR-382-5p**). RT-qPCR confirmed NanoString trends, supporting their selection for functional analyses in granulosa and luteal tissues.

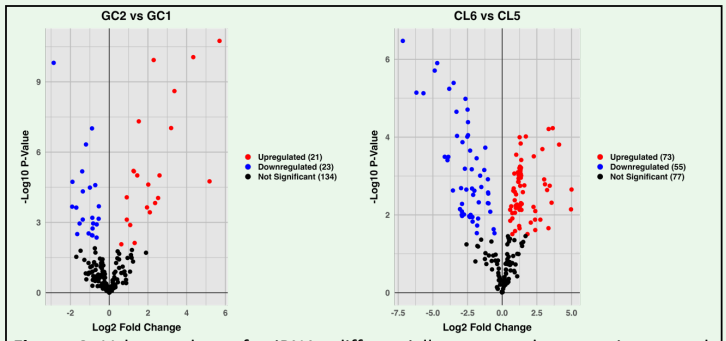

Figure 4: Volcano plots of miRNAs differentially expressed across tissues and timepoints. The strongest shifts occurred in **granulosa (GC) at T2 vs T1 (44 DE miRNAs)** and in **corpus luteum (CL) at T6 vs T5 (127 DE miRNAs)**, highlighting key regulatory transitions during ovulation and luteal development.

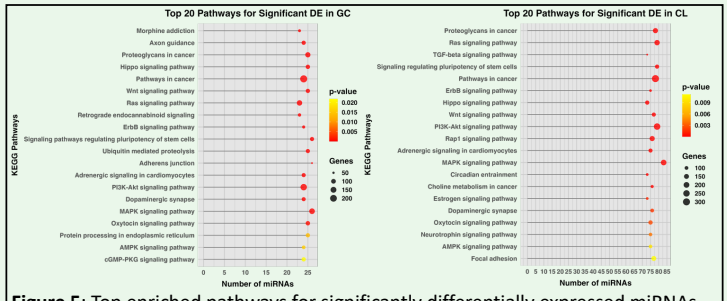

Figure 5: Top enriched pathways for significantly differentially expressed miRNAs.

- Granulosa (GC) at T2 vs T1: **Wnt, MAPK, PI3K–Akt, Hippo, Ras**
- Corpus luteum (CL) at T6 vs T5: **TGF-β, PI3K–Akt, MAPK, Ras, Focal adhesion**

Conclusion

- Regulation of miRNA expression was greatest in granulosa (T2 vs T1) and corpus luteum (T6 vs T5) tissues at ovulation and luteal formation, respectively.
- Four validated candidates (**miR-132-3p**, **miR-144-3p**, **miR-214-3p**, **miR-382-5p**) converge on pathways linked to **steroidogenesis**, **luteal survival**, **angiogenesis**, **and immune regulation**, highlighting their potential as fertility biomarkers in cattle.

Acknowledgments

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