A Conserved Core of Epididymal-Derived microRNAs May Contribute to Modifying the Endometrial Transcriptome to Facilitate Receptivity to Implantation in Placental Mammals

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Placental mammals show a remarkable diversity in sperm morphology, embryo development, implantation strategies and placental structure, among others. The gene networks that regulate these processes can be either conserved, i.e., the same network regulates a fundamental reproductive process, or species-specific and drive diversity. One of the known mediators of gene networks are microRNAs (miRNAs) which can target a multitude of different pathways. There is evidence in some species that the miRNAs acquired by sperm during the epididymal transit play a significant role in female endometrium by facilitating receptivity to implantation; however, whether these miRNAs are conserved in placental mammals has not yet been elucidated.

We hypothesised that a core of conserved epididymal-derived miRNAs may modify the transcriptional landscape in the endometrium to facilitate receptivity to implantation in placental mammals. To test this hypothesis, we accessed publicly available (GEO or publications), epididymal miRNA datasets for representatives of six mammalian families spanning 4 orders, including cow, yak and zebu (*Bovidae* family, 7 data sources), pig (*Suidae*, 5), horse and donkey (*Equidae*, 2), mouse and rat (*Muridae*, 14), guinea pig (*Caviidae*, 1), and human (*Hominidae*, 5). Accessed datasets were generated with RNA-seq or microarray and included epididymal tissue, extracellular vesicles, and sperm. We identified 86 conserved miRNA precursors shared across all families, regardless of the epididymal segment. Of these, 24 precursors were derived from cauda and 69 from caput segment. Interestingly, all 24 cauda precursors were also present in datasets from the caput, indicating potential acquisition of species-specific miRNAs as they transit through the epididymis. These were also detected in ejaculated sperm or seminal plasma from at least one species analysed, suggesting that this set of conserved miRNAs may be the one acting on the endometrium. Therefore, 48 mature miRNAs derived from 24 conserved cauda precursors were used for further analysis. Using RBiomirGS R package, we retrieved overall enrichment for conserved cauda miRNA targets, which showed a broad coverage of cellular functions and pathways by these miRNAs. We next used multiMiR R package to retrieve both predicted and validated targets resulting in over 16,000 unique genes. To assess whether identified conserved cauda miRNAs may target biologically significant transcripts in endometrium, we accessed datasets of endometrial genes that changed their expression in the presence of the conceptus during pregnancy recognition in cow, pig, and horse. Overall, 290 genes were present in the endometrium and responded to conceptus in all three species, out of which, 271 were targets of 38 conserved cauda miRNAs. Using DAVID tool, we found that these 271 endometrial targets were enriched in functional terms related to innate immunity, extracellular matrix, protein ADP-ribosylation, inflammatory response and TNF signalling pathway. Using STRING app in Cytoscape, 237 conserved targets formed a predicted protein-protein interaction cluster, which could be further divided using Markov Cluster Algorithm into

smaller highly interacting groups enriched in: (1) response to stimulus, immune system response and interferon signalling, (2) PPAR signalling, (3) mRNA binding, and (4) Golgi associated vesicle biogenesis. Finally, we built a comprehensive miRNA-mRNA network that can serve as a guide for investigating conserved in-silico interactions of epididymal miRNAs and endometrial targets in placental mammals. Future work will focus on confirming if these predicted conserved miRNAs and their targets modify receptivity to implantation in vitro in species with different implantation strategies.