FROM GENE EXPRESSION TO PREGNANCY PREDICTION: TOWARDS PRECISION ART THROUGH SYSTEMS BIOLOGY AND BAYESIAN MODELING

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Introduction: The implantation window is a limited period during which the endometrium creates the optimal environment for embryo implantation. During this phase, the embryo and endometrium synchronously communicate to enable successful pregnancy [1]. Assisted reproductive technology (ART) require correct identification of the implantation window to increase the chances of embryo implantation following embryo transfer (ET). To investigate and describe the molecular differences between a receptive and a non-receptive endometrium, we analyzed the RNA contained in uterine fluid-derived extracellular vesicles (UF-EVs). EVs are modulators of the microenvironment and mediators of intercellular communication, as their content comes from the cells that produced them [2]. The aim of this study is to identify a transcriptomic profile of the endometrium during the implantation window. Robust graph and bayesian statistical methodologies were used to extract and aggregate RNA-Seq data originating from the sequencing of transcripts contained in UF-EVs to identify clusters of genes that could serve as potential biomarkers of a receptive endometrium.

Objectives: The objective of the research is to characterize and predict the endometrial receptivity leveraging gene expression RNA-seq data of 82 women who underwent ART treatments.

Methods: The patient recruitment and sample processing were performed by the IRCCS San Raffaele Scientific Institute, Milan, Italy. The study included 82 women who underwent blastocyst transfer, whose euploidy was verified through Pre-implantation Genetic Test (PGT). From the follow-up of the patients, it emerged that 37 women achieved pregnancy and 45 women failed to achieve pregnancy after ET. UF-EVs were collected 7 days after the LH peak that marks the beginning of ovulation. Transcriptome sequencing on UF-EVs was performed on both Illumina NextSeq 500 and Illumina NovaSeq 6000. To describe the transcripts information a WGCNA analysis has been performed. Subsequently, to evaluate differences in the transcriptomic profile between women with successful implantation and those with failed implantation after blastocyst transfer, we conducted a differential gene expression (DGE) analysis. To aggregate the information of these transcripts we run a second WGCNA analysis, obtaining 4 modules, which are described thought a over representation analysis, and finally used as input of a Bayesian predictive model.

Results: The resulting RNA-Seq data identifies 14,282 expressed genes that have been analyzed with the WGCNA methodology resulting in 16 modules. After performing a DGE analysis we obtained 966 differentially expressed genes in EVs that have been aggregated in 4 modules by applying a second WGCNA.
Performing an ORA analysis, the most correlated module with pregnancy (M1) (0.40, p-value 2e-04, 95% CI [0.19, 0.56]) contains genes involved in negative regulation of protein kinase and protein phosphorylation. The second module (M2) (cor=0.27, p-value=0.01, 95% CI [0.05, 0.46]) contains transcripts involved in branched-chain amino acid transport, snRNA 3'-end processing, regulation of extracellular matrix disassembly. The third module (M3) (cor=0.33, p-value=0.002, 95% CI [0.12, 0.51]) contains transcripts involved in negative regulation of protein localization, positive regulation of interleukin-8 production, and the fourth module (M4) (cor=-0.27, p-value=0.015, 95% CI [-0.45, -0.05]) contains transcripts of genes involved in negative regulation of DNA-binding transcription factor activity and regulation of telomerase activity. Strongly linearly correlated modules were combined into a single module (M2 + M3).
The extracted WGCNA modules along with the number of previously abortions (PA) and the average size of the vesicles (D90) have been used as regressors in a Bayesian Logistic Regression model reaching a Leave One Out Accuracy of 0.82, Precision of 0.83, Recall of 0.89, F1-score of 0.86 and an AUC of 0.84. Model's results show that the highest odds-ratio (OR) is assigned to the first module (M1) with an OR of 8.2 (90% CI [2.35, 20.98]), the combined module (M2 + M3) has an OR of 4.9 (90% CI [1.40, 10.98]), finally the last module has an OR of 0.05 90% IC [0.03, 0.20]. Previous abortions OR is 0.50, 90% IC [0.27, 0.94] and finally D90 OR is 0.36 90% IC [0.19, 0.81]. All analyses were conducted in R and Stan.

Conclusions: Our findings indicate distinct gene expression differences in the transcript information carried by extracellular vesicles (EVs) between women who achieved a successful pregnancy post-embryo transfer (ET) and those in whom blastocyst implantation did not occur. Rather than focusing on a limited number of significant genes, our analysis revealed a more intricate modulation of transcripts, suggesting the presence of interconnected gene clusters that are either upregulated or downregulated. Studying gene clusters in the endometrium reveals insights into preparing for embryo reception, informing optimal embryo transfer timing and improving ART success. By studying the intricate interplay of gene clusters, our research contributes to a comprehensive understanding of endometrial receptivity, thereby advancing the clinical management of ART procedures. Ultimately, our findings hold the potential to increase the likelihood of successful pregnancy outcomes through improved timing strategies for embryo transfer.

Bibliography

[1] *Giacomini E, Scotti GM, Vanni VS et al. Global transcriptomic changes occur in uterine fluid-derived extracellular vesicles during the endometrial window for embryo implantation. Hum Reprod. 2021;36(8):2249-2274.*

[2] *O'Brien K, Breyne K, Ughetto S, et al. RNA delivery by extracellular vesicles in mammalian cells and its applications. Nat Rev Mol Cell Biol 2020;21:585–522.*