

## **ALTERED TAXONOMIC AND FUNCTIONAL BACTERIAL NETWORKS IN THE ENDOMETRIUM OF WOMEN WITH REPRODUCTIVE FAILURE**

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### **Background**

The endometrial microbiome (EM) significantly impacts reproduction, as dysbiosis in the uterine cavity has been associated with adverse pregnancy outcomes. Here, we applied whole metagenomic sequencing to understand the functional role of the EM in reproductive outcomes, as we hypothesized bacterial genes modulate the uterine environment and human pregnancy.

### **Methods**

Endometrial fluids (n=79) were collected during the window of implantation before personalized embryo transfer. Following clinical outcomes, patients were divided into live birth (LB), biochemical pregnancy (BP), clinical miscarriage (CM), and no pregnancy (NP) groups. Genomic DNA was extracted and sequenced using NovaSeq (Illumina). Host reads were removed by mapping against a human genome reference using BWA. Bacterial profiling was obtained using Diamond. SparCC matrices for network analysis were generated using the SpiecEasi R library. Differences in antibiotic resistance and virulence factors were obtained by mapping with CARD and VFDB databases, differential abundances were reported using DESeq2 library in R.

### **Results**

Taxonomic and functional bacterial networks displayed significant differences between reproductive outcomes; LB and NP displayed unconnected networks with several nodes, while BP and CM showed densely connected networks. *Gardnerella vaginalis* was isolated in LB and NP. In BP, *G. vaginalis* coexisted with *Lactobacillus iners* and both negatively associated with MFS transporter, opposite to *Lactobacillus crispatus*. In CM, *G. vaginalis* displayed a negative association with *L. crispatus* and a positive link with a dense node of genes involved in ABC transporters. Antibiotic resistance genes and virulence factors displayed differential expression between groups, with some genes only found in reproductive failure (RF). Genes highly enriched in RF were related to efflux pumps, antibiotic inactivation, immune modulation, and adherence.

### **Conclusions**

Our findings confirm that changes in EM negatively impact fertility, and suggest bacterial genes related to molecule transport, antibiotic resistance, and immune modulation could be implicated in uterine pathophysiology and RF.