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Title: The Microbiome-Fertility Nexus: Rapid On-Site Long-Read Sequencing for IVF Clinic Diagnostics

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Background: The vaginal microbiome plays a crucial role in reproductive health, yet traditional diagnostic methods for conditions such as bacterial vaginosis exhibit limitations in accuracy and reproducibility. While next-generation sequencing (NGS) has enhanced microbiome analysis, challenges persist regarding cost and turnaround time. This study aimed to establish a rapid and accurate bacterial flora analysis system using the MinION nanopore sequencer and apply it to vaginal microbiome analysis.

Methods: We optimized a protocol for full-length 16S rRNA gene sequencing using MinION. The system was rigorously validated using mock bacterial communities and fecal samples, with results compared to Illumina MiSeq sequencing. We then applied this method to analyze vaginal microbiome samples, evaluating sampling techniques, read depth requirements, and Lactobacillus species identification accuracy.

Results: MinION-based full-length 16S rRNA sequencing demonstrated high accuracy in identifying bacterial species in both mock communities and complex fecal samples. Improved primers significantly enhanced the detection and quantification of Bifidobacterium species, addressing a previous limitation of MinION-based approaches. In vaginal samples, swab and lavage sampling methods yielded comparable results. Analysis of 3,000 reads, obtainable within approximately 10 minutes of sequencing, proved sufficient for comprehensive vaginal microbiome profiling. Full-length (V1-V9) 16S rRNA analysis showed superior accuracy in Lactobacillus species identification compared to partial (V3-V4) sequencing.

Conclusions: We successfully established a MinION-based bacterial flora analysis system enabling rapid (within 2 days) and accurate vaginal microbiome profiling. This method overcomes limitations of traditional diagnostic approaches and the constraints of conventional NGS platforms. Our system holds promise for clinical applications, particularly in reproductive medicine, where timely microbiome data could inform treatment decisions.

Future Directions: A prospective study investigating the relationship between vaginal microbiome composition and infertility treatment outcomes using this novel MinION-based analysis system is currently underway.