RESEARCH TOPIC CLI10

Beyond the Surface: Metagenomic-Driven Profiling of the Endometrial Microbial Environment via Embryo Transfer Catheters (VIMET-CAT Study)

Research area

Surgical Area

Clinical Unit name

Department of Biomedical Sciences, Humanitas University, Pieve Emanuele

Gynecology and Reproductive Medicine, Fertility Center, Humanitas Research Hospital, Rozzano

Supervisor

Prof. Paolo Emanuele Levi Setti paolo.levi_setti@hunimed.eu

Co- Supervisor

Dr. Federico Cirillo federico.cirillo@humanitas.it

Prof. Valeria Cento valeria.cento@hunimed.eu

Abstract

State of the art

Dysbiosis of the endometrial microbiome has been related to pathological conditions such as chronic endometritis and infertility. Researchers, interested in the study of women's infertility, decided to assess a potential role of the endometrial microbiome in the reproductive outcomes, demonstrating the existence of an endometrial microbiome that is highly stable during the acquisition of endometrial receptivity and associating pathological modification of its profile with poor reproductive outcomes. While the bacterial profile has been widely studied, there is a lack of evidence about the virome, and in particular the presence of HPV in the endometrial cavity and its possible consequences in reproductive outcomes.

Research question and project aims:

The main objective is to analyze the composition and functional potential of the endometrial microbiome and virome, including HPV, in the site of implantation in relation with reproductive outcomes in women undergoing fertility treatments.

Secondary aims are:

- to establish a standardized pipeline for metagenomic profiling in the setting of infertility.
- to establish a longitudinal metagenomic profiling approach to identify stable microbial biomarkers (bacterial and viral) predictive of endometrial receptivity and successful implantation.
- To investigate the interactions between bacterial and viral communities in the endometrial microbiome, and how these interactions influence immune modulation and endometrial receptivity.

This platform will enable high-resolution, multi-kingdom (bacteria, viruses, fungi, archaea) characterization of the endometrial microbiome for comprehensive profile and further development of predictive analytic pipelines.

Research design:
Population and selection criteria:
500 infertile women undergoing embryotransfer of a single cryopreserved blastocyst after in vitro fertilization (IVF) or Intracitoplasmatic Sperm Injection (ICSI) at the ICH Fertility Center
Inclusion criteria:
- Women undergoing Single Embryo Transfer (SET) at blastocyst stage.
- Age ranging from 18 to 44 years old.
- Body Mass index (BMI) ranging from 18 to 30 kg/m2.

Exclusion criteria:
- Women undergoing PGT analysis.
- Women affected by Endometriosis.
- Pap smear positivity.
- Presence of sactosalpynx or a known pelvic infection.
- Wide range antibiotic therapy in the previous month.

Overall methodology, methods
Metagenomics profiling at the Implantation site:
After the embyotransfer procedure, the distal 5-mm portion of the catheter tip are cut and sterilely placed in a DNA-free PCR tube. The samples collected are processed to perform cell lysis and genome extraction, followed by creation of metagenomic libraries that are screened by Illumina platform.
Bioinformatic Data Analysis
Analysis of sequencing data are performed with bioinformatic tools to simultaneously identify and characterize the microbiomal community.
The simultaneous analysis of viruses, fungal and the bacterial microbiota enables the elucidation of differences in the inter-kingdom equilibrium and will allow us to determine the endometrial microbiota composition in the site of potential embryo implantation.
A specific subanalisys will included only patients with reapeted transfer with negative result.

Ethical Considerations
All women enrolled in this study will sign a written informant consent form, previously validated by ICH Ethical Board Committee. The sample collection will not alter any part of the clinical practice. It is worthwhile pointing out that we will not touch anyhow any human embryo. Our exclusive focus will be on the endometrial cells that will adhere on the catheter tip. In standard IVF/ICI procedures, the catheter used for embryo transfer is normally discarded after the transfer procedure.

Expected impact
- Unprecedented scale of sample analysis. Leveraging access to a large and diverse cohort of women undergoing fertility evaluation, the study will analyze an unprecedented

Humanitas University – Via Rita Levi Montalcini, 4 – 20072 Pieve Emanuele (MI) Italy
CF 97692990159 – Tel. 02.8224.3777 – info@hunimed.eu – hunimed.eu

number of endometrial samples. This scale will allow for robust statistical power, the identification of rare microbial signatures, and the stratification of patients based on microbial and clinical phenotypes.

- Identification of microbial biomarkers. Specific bacterial and viral taxa—including HPV genotypes—associated with endometrial receptivity, chronic endometritis, and infertility could be used as biomarkers for diagnostic, prognostic, or therapeutic purposes in reproductive medicine.
- Integration of the virome into reproductive microbiome studies. By incorporating viral metagenomics, the study will provide the first in-depth characterization of the endometrial virome, shedding light on the prevalence, diversity, and potential pathogenic roles of viruses such as HPV in the uterine environment.
- Predictive models for reproductive success. Using machine learning and integrative bioinformatics, the project will develop predictive models that correlate microbial profiles with implantation success, pregnancy rates, and IVF outcomes, offering new tools for personalized reproductive care.
- Standardized protocols and open-access resources. The project will generate standardized protocols for sample collection, sequencing, and data analysis, along with open-access databases and analytical pipelines. These resources will support future research and clinical translation, fostering collaboration and innovation in the field.

Scientific references

1. The Implication of The Vaginal Microbiome in Female Infertility and Assisted Conception Outcomes. Chen X, Sui Y, Gu J, Wang L, Sun N (Genomics Proteomics Bioinformatics. 2025)

2. The impact of the female genital tract microbiome in women health and reproduction: a review. Punzón-Jiménez P, Labarta E. (J Assist Reprod Genet. 2021)

3. Vaginal microbiota are associated with in vitro fertilization during female infertility. Wang T, Li P, Bai X, Tian S, Yang M, Leng D, Kui H, Zhang S, Yan X, Zheng Q, Luo P, He C, Jia Y, Wu Z, Qiu H, Li J, Wan F, Ali MA, Mao R, Liu YX, Li D. (Imeta. 2024)

4. Human genital tracts microbiota: dysbiosis crucial for infertility. Venneri MA, Franceschini E, Sciarra F, Rosato E, D'Ettorre G, Lenzi A.(J Endocrinol Invest. 2022)

5. Increased Risk of Infertility in Women Infected with Human Papillomavirus. Pebdeni PH, Saffari F, Mollaei HR, Mirshekari TR, Sadat RH, Habibzadeh V, Saeed L, Soodejani MT, Ahmadrajabi R. (J Reprod Infertil. 2023)

6. Human papillomavirus infection and female infertility: a systematic review and meta-analysis. Yuan S, Qiu Y, Xu Y, Wang H. (Reprod Biomed Online. 2020)

7. 2020 WHO Classification of Female Genital Tumors. Höhn AK, Brambs CE, Hiller GGR, May D, Schmoeckel E, Horn LC. (Geburtshilfe Frauenheilkd. 2021)

8. ESHRE Guideline Group on Viral infection/disease; Mocanu E, Drakeley A, Kupka MS, Lara-Molina EE, Le Clef N, Ombelet W, Patrat C, Pennings G, Semprini AE, Tilleman K, Tognon M, Tonch N, Woodward B. (Hum Reprod Open. 2021)

9. Testing for Human Papillomaviruses in Urine, Blood, and Oral Specimens: an Update for the Laboratory. Poljak M, Cuschieri K, Alemany L, Vorsters A. (J Clin Microbiol. 2023)

10. Assessing the performance and utility of targeted next-generation sequencing for screening and genotyping of human papillomaviruses. Lee JH, Park YM, Kim J, Kwon GC, Kim S. (Diagn Microbiol Infect Dis. 2024)

Type of contract

Two PhD scholarship of € 22.400 gross per year awarded by Humanitas University. This sum is exempt from IRPEF income tax according to the provisions of art. 4 of Law no. 476 of 13th August 1984 and is subject to social security contributions according to the provisions of art. 2, section 26 and subsequent sections, of Law no. 335 of 8th August 1995 and subsequent modifications.

Due borse di dottorato pari a € 22.400 annui lordi erogata da Humanitas University. Importo non soggetto a tassazione IRPEF a norma dell'art. 4 della L. 13 agosto 1984 n. 476 e soggetto, in materia previdenziale, alle norme di cui all'art. 2, commi 26 e segg., della L. 8 agosto 1995, n. 335 e successive modificazioni.

Humanitas University – Via Rita Levi Montalcini, 4 – 20072 Pieve Emanuele (MI) Italy
CF 97692990159 – Tel. 02.8224.3777 – info@hunimed.eu – hunimed.eu