STUDY PROTOCOL

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Microbiome and Metabolome Profiles in Couples Undergoing IVF and Their Association With Reproductive Outcomes: A Prospective Cohort Study

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1. Official Title

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2. Document Date

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3. Investigators and Affiliations

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4. Funding

This study is supported by the Swedish Research Council, the Swedish Cancer Society, Radiumhemmets Research Funds, Stockholm Region ALF grants, and Karolinska Institutet.

5. Conflicts of Interest

The investigators declare no conflicts of interest.

6. Background and Rationale

Infertility affects a substantial proportion of couples worldwide, and assisted reproductive technologies, including in vitro fertilization, are increasingly used to achieve pregnancy. Despite advances in IVF, implantation failure, early pregnancy loss, and adverse obstetric outcomes remain insufficiently understood.

Emerging evidence suggests that the human microbiome may influence reproductive physiology through immune, inflammatory, and metabolic pathways. The female reproductive tract microbiome has been associated with implantation success and miscarriage risk, while the gut microbiome may contribute to systemic immune regulation through microbial metabolites. The potential contribution of the male reproductive microbiome to reproductive outcomes is less well characterized.

Most existing studies are cross-sectional, focus primarily on women, or evaluate a single biological compartment. Prospective longitudinal studies integrating microbiome, metabolome, and immune markers in couples undergoing IVF remain limited.

This study is designed to evaluate microbiome and metabolome profiles in couples undergoing IVF and to assess their association with reproductive outcomes.

7. Objectives

7.1 Primary Objective

To evaluate the association between baseline microbiome and metabolome profiles and live birth following IVF treatment.

7.2 Secondary Objectives

1. To evaluate associations between microbiome and metabolome characteristics and time to pregnancy.
2. To assess relationships between microbial profiles and fertilization rate, embryo development, implantation success, and clinical pregnancy.
3. To investigate associations between microbiome signatures and miscarriage.
4. To evaluate associations between microbiome and metabolome profiles and obstetric outcomes.
5. To explore the contribution of the male reproductive microbiome to IVF outcomes.

8. Study Design

This study is a prospective observational cohort study of couples initiating IVF treatment in Stockholm County, Sweden. Biological samples will be collected at baseline before initiation of IVF stimulation and, when applicable, during early pregnancy according to the study sampling plan. Couples will be followed from initiation of IVF treatment until pregnancy or for up to 1 year, whichever occurs first. Pregnancies achieved during follow-up will be followed until completion, including miscarriage or live birth.

Because this is an observational cohort study, no experimental intervention will be assigned as part of the protocol.

9. Study Population

9.1 Inclusion Criteria

- Women aged 18 to 40 years undergoing IVF treatment
- Male partners aged 18 to 56 years
- Ability to provide informed consent
- Ability to complete study questionnaires

9.2 Exclusion Criteria

- Pregnancy at the time of enrollment
- Systemic antibiotic use within the previous 4 weeks
- Active genital infection at the time of sampling

10. Sample Size

The study aims to enroll 500 couples undergoing IVF treatment. In addition, 10 sperm donors and 10 oocyte donors will be included as reference groups for microbiome comparisons.

11. Study Procedures and Biological Sampling

11.1 Female Participants

- Rectal swab
- Vaginal swab
- Peripheral blood sample

11.2 Male Participants

- Semen sample
- Rectal swab

11.3 Timing of Sample Collection

For the main IVF cohort, samples will be collected at baseline before initiation of IVF stimulation. Additional sample collection may occur during early pregnancy, when applicable and according to the study sampling plan.

12. Microbiome and Metabolome Analyses

Microbial composition will be characterized using shotgun metagenomic sequencing to identify bacterial, viral, fungal, and archaeal communities. Metabolomic profiling will be performed using mass spectrometry-based analytical platforms.

Sequencing data will undergo quality control procedures, removal of human reads, and taxonomic classification using established bioinformatics pipelines.

Immune marker analyses in blood samples will be performed using laboratory-based immunologic assays, as specified in the analytical plan.

13. Data Sources

- Participant questionnaires
- Clinical IVF records
- Biological sample analyses
- Linkage with Swedish national health registers, including the Swedish Pregnancy Register

14. Outcomes

Planned study outcomes include reproductive and laboratory outcomes such as:

- Live birth
- Time to pregnancy
- Fertilization rate
- Embryo development
- Implantation success
- Clinical pregnancy
- Miscarriage
- Obstetric outcomes
- Female and male microbiome profiles
- Metabolomic profiles
- Immune marker levels in female blood samples
- Semen parameters, including sperm concentration

Detailed outcome titles, descriptions, and time frames should be entered in the ClinicalTrials.gov record in the Outcome Measures section.

15. Statistical Analysis

Descriptive statistics will be used to summarize participant characteristics.

Associations between microbiome and metabolome profiles and reproductive outcomes will be evaluated using multivariable regression models adjusted for relevant confounders, including maternal age, body mass index, smoking status, infertility diagnosis, and medication exposure.

Microbiome diversity metrics, including alpha diversity and beta diversity, will be calculated. Differential abundance analyses will be performed using compositional statistical approaches with correction for multiple testing.

Exploratory analyses may assess relationships between microbiome composition, metabolomic profiles, immune markers, and reproductive outcomes.

16. Ethical Considerations

The study has been approved by the Swedish Ethical Review Authority, Dnr 2024-07146-02.

Written informed consent will be obtained from all participants before enrollment.

No names or identifying details of research participants are included in this uploaded protocol document.

17. Dissemination

Results will be disseminated through scientific conferences and peer-reviewed publications.

18. Graphic Abstract of the Protocol