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Title: The amount of bacterial DNA constituting the endometrial microbiome could affect pregnancy efficacy; a single center prospective cohort study

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Introduction: Endometrial microbiota, which differ from those in other body regions, play pivotal roles in embryo implantation, endometriosis, infections, and carcinogenesis, as revealed by recent advances in non-culture analysis methods. This study examines the influence of bacterial DNA abundance on infertility outcomes. **Methods:** We prospectively analyzed 499 cases out of 530 that met the inclusion criteria between August 2019 and July 2021. Criteria included two consecutive failed embryo transfers with high-quality embryos, possession of at least one high-quality embryo at study entry, non-smoking habits in both partners, and consent to study participation. Endometrial biopsies were performed in the mid-luteal phase with microbiome analysis performed according to Igenomix Japan's protocol. **Results:** Significant differences between groups were noted in BMI and AMH levels ($p=0.02$, 0.004 , respectively). Logistic regression analysis showed age and ultra low biomass were associated with poorer pregnancy outcomes ($OR=0.88$, $95\%CI=0.84-0.93$; $OR=0.28$, $95\%CI=0.08-0.91$). Kaplan-Meier estimation revealed significant differences ($p=0.0049$) in the time to pregnancy among microbiome groups, with the ultra low biomass group showing the worst prognosis. This outcome was also observed in no-Lactobacillus cases ($p=0.03$). **Discussion:** The cause of extremely low endometrial microbiome cases remains speculative, with potential influences including technical issues during specimen collection, microbiota analysis limitations, personal microbiota, abnormal uterine morphology, and sexual intercourse frequency. Moreover, the endometrial microbiome, though less dense and more hypoxic than the vaginal microbiome, has unique metabolites that could influence implantation. **Conclusion:** Pregnancy rates may be negatively affected by ultra-low endometrial microbiome biomass. Additionally, antibiotics and/or probiotics could potentially improve fertility outcomes even when the Lactobacillus spp. percentage is low ($<90\%$). Further research is needed to understand these implications fully.