27th ECCMID Vienna, Austria 22 – 25 April 2017
The congress of ESCMID

Session: P090 Sexually transmitted infections

Category: 2d. Abdominal/gastrointestinal, urinary tract & genital infections

25 April 2017, 12:30 - 13:30
P1880

Abnormal vaginal microbiota affect the reproductive outcome in in vitro fertilization (IVF) patients

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Background: Based on recent evidence, the endometrium is not sterile. In contrast the endometrium harbors a polymicrobial microbiota that share close similarity to the vaginal microbiota. The endometrial microbiota might have an important impact on the implantation of the embryo and subsequently the reproductive outcome. However, only a few studies have investigated this emerging issue. The aim of the present study was to investigate vaginal specimens with 16S rRNA gene sequencing compared to the qPCR approach, developed in a prior publication, to detect IVF patients at risk for poor reproductive outcome.

Material/methods: Briefly, a total of 130 infertile women (90% Caucasian) attending IVF treatment at two centers in Denmark were prospectively included at their first consultation prior to IVF treatment. During speculum examination, vaginal samples were taken from the posterior fornix with Copan Eswab™. Furthermore, a vaginal smear was gram stained for microscopy and Nugent scoring for bacterial vaginosis (BV), Gold standard. Following DNA extraction, qPCR and 16S rRNA gene sequencing was performed on the specimens. Primers targeted the V4 region and were similar to the ones in the earthmicrobiome project.

Results: The mean level of reads from *G.vaginalis* and *A. vaginae* in Nugent score BV was 63 (95%CI 36-112) times higher compared to Nugent normal group. The area under the ROC curve between BV and the sum of *G.vaginalis* and *A. vaginae* from 16s rRNA sequencing was 0.94 (95%CI 0.93-0.95). However, the interrater variability is high with only 66% agreement, which is mainly contributed to *Lactobacillus iners*, P=0.02. In a principal component analysis, a total of 28/32 qPCR abnormal vaginal microbiota (AVM) positive samples were clustered together. We could confirm that qPCR normal group had a significantly higher proportion of *Lactobacillus spp.* compare to the AVM qPCR group which had significantly higher proportions of *Gardnerella vaginalis* and *Atopobium vaginae*. Regarding

the reproductive outcome, the 16S AVM group were also able to predict a significantly higher risk of a poor reproductive outcome regarding both clinical pregnancy (P=0.02) and live birth (P=0.02).In a broader panel of tentative pathogenic bacteria including *Streptococcus spp., Prevotella spp., Gardnerella vaginalis*, *Atopobium spp., Sneathia sanguinegens*., no significant difference in live birth could be observed between AVM (broad panel) and normal vaginal microbiota, P=0.11.

Conclusions: The qPCR assay, aiming for *Gardnerella vaginalis* and *Atopobium vaginae,* is an objective, robust and low-cost diagnostic tool non-inferior to 16s rRNA gene sequencing to provide the clinician with an easy access yes/no diagnosis of AVM. However, there is a need for corroboration to further establish the correlation between AVM and poor reproductive outcome.