Evaluation of the female reproductive tract microbiome and innate immune profile in endometriosis

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INTRODUCTION

Emerging evidence suggests the presence of a distinct endometrial microbiome. Little is known about how commensal microbiota interact with the host immune system, or how they may be involved in host defence. There has also been a suggestion of a specific microbial composition in the female reproductive tract (FRT) in endometriosis, with no conclusion in the literature to date on specific composition.

While next generation 16S or shotgun sequencing has been used most frequently to evaluate the microbiome at various sites, a quantitative PCR (Q-PCR) approach offers a quicker, cost effective alternative.

The objective of this study was to evaluate the presence of specific bacterial phyla along the FRT using a Q-PCR approach, to compare the findings in women with and without endometriosis, and to correlate with endometrial Antimicrobial Peptides (AMPs), an important component of the innate immune system.

Table 1: Patient Characteristics

	Endometriosis	No endometriosis	p value
	n=9	n=4	
	Mean (± SD)		
Age (years)	33 (± 3.5)	37.24 (± 4.43)	0.0878
AMH (pmol/L)	15.57 (± 9.92)	17.72 (± 6.12)	0.6991
BMI (kg/m²)	22.37 (± 1.92)	23.16 (± 3.65)	0.6251
	Number (%)		
Nulliparous	7 (77.78%)	2 (50%)	0.5301
Multiparous	2 (22.22%)	2 (50%)	

METHODS

This was a prospective cohort study. Patients attending an infertility clinic were recruited.

Endometrial fluid samples were aspirated using an embryo transfer catheter, in the cycle prior to ART or at the time of laparoscopy. Endometrial biopsy samples were also taken. Peritoneal fluid was aspirated at laparoscopy.

DNA was extracted and a Q-PCR approach used to evaluate for bacterial phyla and Lactobacillus genus. Positive and negative controls were used. RNA was extracted from the endometrial tissue samples, and genes for specific AMPs examined via Q-PCR.

Correlation between microbiota and endometrial AMPs was performed using Spearman's correlation coefficient.

RESULTS

Thirteen patients were included in the study (see *Table 1*). Bacterial DNA was isolated from 11 of the 12 endometrial fluid samples tested (*Figure 1*). Of the 6 patients who had peritoneal fluid samples taken, bacterial phyla were identified in 5 cases. The most abundant phylum isolated was Firmicutes. Firmicutes DNA was identified in 11 of the 12 endometrial samples. Lactobacillus genus was expressed in the endometrial fluid of 7 of 12 patients tested (58.33%).

The distribution of bacteria was different in the endometrium and peritoneal cavity. There was also a difference seen between the endometrium and lower FRT (*Figure 2*), suggesting that the upper FRT microbiome may be a distinct and separate community to that of the lower FRT.

There was a difference in pattern of expression of bacteria in endometriosis compared to controls (*Figure 3*), but this did not reach significance. There was a significant negative correlation between both S100A9 and SLPI AMPs and the Lactobacillus genus (*Figure 4*).

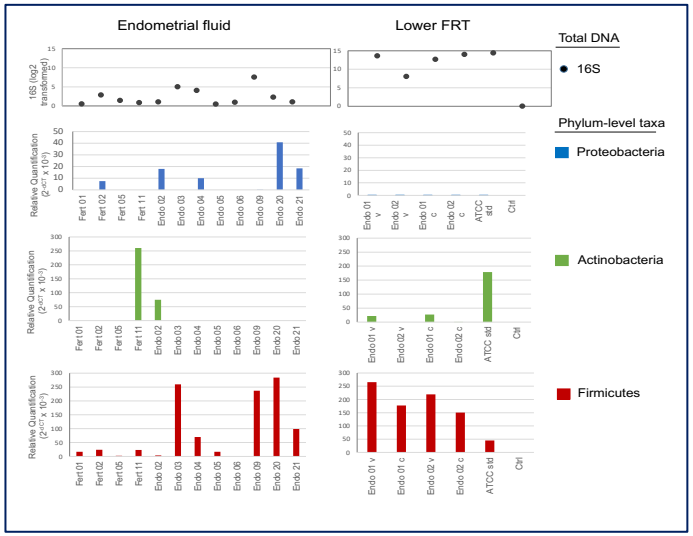

Figure 1: Bacterial phyla in the FRT

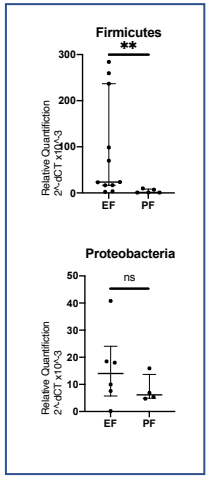

Figure 2: Bacterial phyla in endometrial (EF) Vs peritoneal fluid (PF)

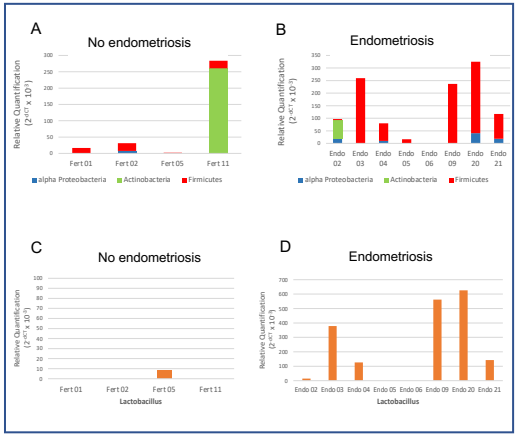

Figure 3: Bacteria in endometrial fluid in endometriosis. Differences in bacterial phyla (A and B) and Lactobacilli (C and D) are shown between those with endometriosis and those without

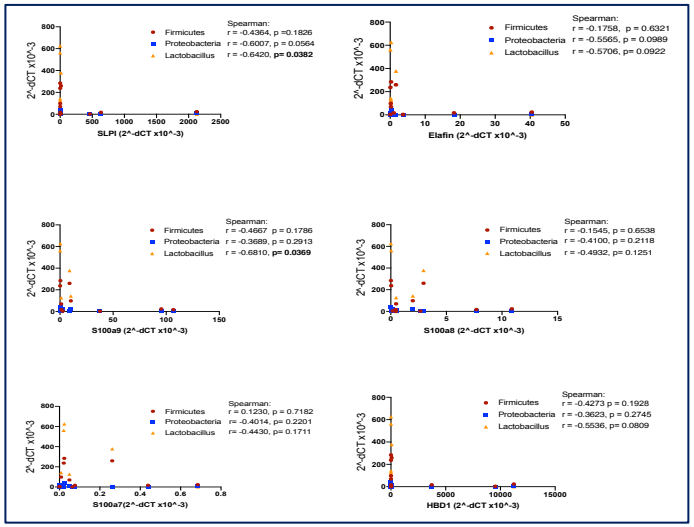

Figure 4: Correlation of AMPs and microbiota.

CONCLUSION

In this study we found that bacteria are detectable via Q-PCR in the very low biomass compartments of the endometrial and peritoneal cavities. In keeping with many other studies of the FRT microbiome, the most abundant bacterial phylum was Firmicutes.

We showed a potential difference in microbial composition in the endometrium in endometriosis patients compared to controls, but this did not reach statistical significance, possibly due to the small numbers of subjects. We also noted a significant negative correlation between the relative abundance of both S100A9 and SLPI AMPs and the Lactobacillus genus.

These findings warrant further investigation in order to further our knowledge of the endometrial microbial and immune environment in endometriosis, with the hope of identifying microbial or immune markers in endometriosis which may lead to novel diagnostic and treatment methods.

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