Bacterial vaginosis (BV) is a common vaginal disorder of women of reproductive age. It is commonly accepted that the microbial switch from normal to BV state is characterized by a decrease in vaginal colonization by Lactobacillus species together with an increase in the number of Gardnerella vaginalis (GV) and other anaerobes.

GV can form a biofilm on vaginal epithelium and this appears to be involved in the pathogenesis of BV and in antibiotic failure. GV biofilms may also facilitate growth of other anaerobes. We hypothesized that an important role for vaginal lactobacilli in the maintenance of vaginal health is prevention of biofilm formation. Further research into the GV interactions with other anaerobes may be crucial to understand BV etiology.

Therefore, our goal was to study competitive initial adhesion between L. crispatus and several BV anaerobes. Also, this study aimed to evaluate differences in biofilm formation between a GV strain from a healthy subject (5-1) and the other anaerobes as compared with a BV GV strain (101). In addition to GV strains, P. bivia, F. nucleatum, M. mulieris and A. vaginae were used in this investigation of bacterial social interaction.

### Methods

#### Initial adhesion competitive assays through FISH analysis

1. Initial adhesion at 100 rpm for 30 min was conducted in glass 8-well slides, using an equal mixture of L. crispatus 39G and an anaerobe (GV 5-1 and 101, P. bivia, F. nucleatum, M. mulieris and A. vaginae) at the same concentration (10^7 CFU/ml);
2. Each well was washed with PBS and fluorescently stained with a lactobacilli specific probe and DAPI;
3. Bacteria counting of the adhesion was done by 20-fields enumeration in each well.

#### Biofilm formation assays through quantitative PCR analysis

1. Mixed biofilm formation between a pre-established (24h) GV strain biofilm and a second inoculation by other anaerobe until reached 48h incubation (37°C at 5%CO2);
2. Each well was washed with PBS and DNA extraction was done by an enzymatic kit;
3. Quantitative PCR was realized with specific primers for each bacteria for 40 cycles.

#### Results

**Table 1** - Results of the quantitative PCR (qPCR) from mixed biofilm formation assays with Gardnerella vaginalis strains (5-1 and 101) and a second BV anaerobe. Legend - CT, threshold cycle; Tqv 5-1, G. vaginalis 5-1; Gv 101, G. vaginalis 101; Mm, M. mulieris; Av, A. vaginae; Pb, P. bivia; Fu, F. nucleatum. All the experiments were realized in triplicate.

### Conclusions

Our results suggest that G. vaginalis 5-1 and 101 strains had the greatest initial adherence capability in presence of an equal number of L. crispatus cells, followed by P. bivia, M. mulieris and A. vaginae.

In addition, qPCR analysis of the two species biofilms revealed that both G. vaginalis strains established commensal relationships with all other anaerobes except with P. bivia, for which a synergistic relation was found. In fact, a synergistic relationship between these two species had previously been noted. Importantly, G. vaginalis 101 (pathogenic strain) showed nearly a 2-fold increase in biofilm formation when compared with G. vaginalis 5-1 (healthy strain) in the presence of any other anaerobe studied.

In conclusion, this study revealed potentially relevant differences between the adherence and biofilm formation of BV and healthy G. vaginalis isolates in the presence of L. crispatus and other BV well-known anaerobes. Further research in these GV strains and their social interactions with other BV anaerobes may be crucial for better understanding of the BV etiology.