Bacterial interactions in the female genital tract

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SUMMARY

This thesis examines and describes interactions between pathogens, microbiota, and the host as they happen in the female genital tract. Additionally, it looks into the effects of these interactions on the risks of being affected by infections, disease, and fertility complications. In **Part 1** a specific focus is put on the interactions of invading pathogens and the immune system of the host, while in **Part 2** the relations between the vaginal microbiota, potential pathogens, and the host's reproductive health are further examined.

There is often a large discrepancy between patients in their clinical presentation while having the same infection in the vaginal tract. This discrepancy is most noticeable in the difference between symptomatic and asymptomatic patients. Although there are many potential reasons for the different presentation of these diseases between patients (e.g. diet, physical health, smoking, the composition of the vaginal microbiota), in **Part 1** we focus on the immune response as it is produced by the host against invading pathogens. This is done by studying potential factors influencing the ability of the host to effectively deal with the invading pathogen. The genetic makeup of the host may not always accurately reflect his or her potential of fighting of an infection. Therefore, we look at the variation in expression of cytokines upon infection between patients. The levels of actually expressed cytokines give a more direct picture of this potential and how this affects the clinical presentation of the most common bacterial sexually transmitted infections (STI).

The vaginal microbiota is an integral part of the health of the female genital tract. Its immunological function has been clearly defined as a capable mechanical barrier warding off opportunistic pathogens. However, the study of complex interactions between the microbiota and outside factors has clarified multiple other roles in the health of the host. **Part 2** of this thesis first describes current knowledge of these complex interactions between the microbiota and *Chlamydia trachomatis* and *Mycoplasma genitalium*. Additionally, the extent of the effect the vaginal microbiota has on in vitro fertilisation (IVF) during various stages of pregnancy is described.

Furthermore, it describes the vaginal microbiota composition of subfertile women. Finally, to clarify the extent of anatomical coverage of the vaginal microbiota a comparison is made between these vaginal microbiota compositions and microbiota found after sampling urine from the same person.

Part 1: Pathogens in the vaginal tract and their interaction with the immune system

Analysing the expression of cytokines is the most direct way of studying the extent of an immune response to an infection. Host specific differences in cytokine expression can directly lead to differences in the progression and clinical presentation of bacterial STIs. Conversely, bacteria specific differences can also lead to an increased cytokine expression in the host. In **Chapter 1** we perform meta-analyses on the available literature covering the expression of cytokines during infection with *C. trachomatis*, *Neisseria gonorrhoeae*, and *Treponema pallidum*. The results of these meta-analyses show that increases in interleukin (IL)-1, IL-6, IL-8, IL-10, tumor necrosis factor α (TNFα), or interferon (IFN)-γ significantly affect the clinical outcome of *C. trachomatis* infections. All of these effects, except for the ones caused by increased IL-10 expression, caused more severe infections with *C. trachomatis*. Interestingly, an increase in *C. trachomatis* linked complications such as tubal pathology and pelvic inflammatory disease was also found in people that had an increased expression of IFN-γ and TNFα. An increased expression of IL-10 had reduced the severity of the infection, which is in line with the immune-regulatory function of this cytokine. Similarly to *C. trachomatis*, multiple studies into IL-1 and IL-8 expression during *N. gonorrhoeae* infection showed aggregated effects that significantly increase the severity of the disease. Unfortunately, these results could not be linked to specific late complications of *N. gonorrhoeae* infections. Lastly, for *T. pallidum* infection, aggregated study data showed that IFN-γ expression variation in hosts could be linked to an increase in severity of this disease. Most notably, the increased expression of this cytokine facilitated the progression of the disease into neurosyphilis, which is an extremely damaging and potentially lethal stage of this disease. An important limitation found in the meta-analyses was that multiple potentially relevant cytokines could not be included in a meta-analysis, as not enough studies into the described infections had included the expression levels of these cytokines.

Expression of inflammatory cytokines is for a large part initiated through the NF-κB pathway. Toll like receptor (TLR) 9 is one of the pathogen recognition receptors (PRR) that triggers the NF-κB pathway after binding with unmethylated CpG DNA. In **Chapter 2** we use the previously shown fact that the hexameric configuration of these CpG DNA motifs has either an increased or reduced stimulating effect on the activation of the NF-κB pathway through TLR9. Using an *in silico* setup, we calculate the CpG index of 11 bacterial species which are commonly found in the vagina in either a pathogenic role or as commensal bacteria. Most notably, *N. gonorrhoeae* shows a CpG index of -79.5 and is the only included bacterial species

to show an inhibitory effect. *G. vaginalis* showed the highest CpG index with a value of 26.2. *Lactobacillus* species showed a near neutral CpG index value of 4.2, which may be linked to the commensal role of this species in the vagina. However, no link between CpG index values and pathogenicity or commensality could be found.

Having covered the genetic makeup of bacterial DNA in the previous chapter, **Chapter 3** discusses genetic differences between hosts. The characteristic symptom of an infection with *Haemophilus ducreyi* are painful genital ulcers known as chancroids. However, the risk of development of these ulcers differs per person, with some infected hosts remaining asymptomatic. This suggests a genetic factor in the severity of this disease. In this chapter 105 volunteers residing in the USA are infected at 3 sites on the arm with *H. ducreyi*. The number of developed ulcers is then taken as a measure of severity of the disease. Single nucleotide polymorphism (SNP) analyses on the host DNA, targeting 14 SNPs related to 7 PRR pathway related genes, created a link between the severity of the infection and the host genetics. In European American volunteers, the SNP *TLR9* +2848 GG has a protective effect on the severity of the disease, and the *TLR9* TA haplotype of *TLR9* -1237 and *TLR9* +2848 has a risk-enhancing effect. Results differed in African American volunteers, where a protective effect was found for *IL10* -2849 AA, and a risk enhancing effect was found for the *IL10* -2849 A, *IL10* -1082 A, and *IL10* -819 C haplotype. Larger studies into the effect of host genetic differences could discover even more associations during infection with *H. ducreyi*, as a clear link between host Immunogenetics and infection severity has now been made apparent.

Part 2: The interaction between the vaginal microbiota and host reproductive health

The interactions between the vaginal microbiota and pathogens are a crucial initial step in the host susceptibility to a disease. The current state of knowledge regarding these interactions between the microbiota and *C. trachomatis* as well as *M. genitalium* are reviewed and discussed in **Chapter 4** of this thesis. The role of oestrogen as a host factor with indirect effects on the microbiota and pathogen interactions is of specific interest. Other points of discussion include the effects of lactic acid levels and H_2O_2 on *C. trachomatis* and *M. genitalium*. Lactic acid has been shown to be a competent inhibitor of *C. trachomatis*, while H_2O_2 inhibits bacterial vaginosis (BV) associated bacteria such as *M. genitalium*. Generally though, the presence of a large abundance of *Lactobacillus spp.* in the vagina is an indicator for reduced susceptibility to either of the studied pathogens. A notable result is the

identification of the gap in current knowledge regarding interactions between *M. genitalium* and the vaginal microbiota. *C. trachomatis* is extensively studied, however for *M. genitalium* we often needed to examine the mechanisms related to other *Mycoplasma* species to derive answers. Additionally, interactions of *M. genitalium* with the vaginal microbiome and the host often need to be speculated about through their functionality with other pathogens, such as *C. trachomatis* in the case of this chapter.

The relation between vaginal microbiota and early pregnancy development process during IVF treatment is discussed in **Chapter 5**. Due to the timing, the potential effect of the vaginal microbiota in this early pregnancy process is mostly apparent during the conception. A meta-analysis aggregates six studies to show that the presence of abnormal vaginal microbiota, represented as BV, leads to 1.4 times as much likelihood for IVF failure in this early stage of pregnancy. However, during the systematic review of literature, it has become clear that methodologies in studies examining the vaginal microbiota during IVF treatment often varies at important points. For instance, microbiota profiles are likely to differ between studies if the vaginal sample is taken at the same time as an antibiotic treatment compared to no antibiotics. This chapter suggests a number of methodological factors that deserve specific attention from readers and researchers interested in the topic of microbiota during IVF.

In **Chapter 6** we ask whether the vaginal microbiota of a woman undergoing IVF treatment can give an indication about the outcome of the IVF treatment. Current success rate of IVF treatments is close to 30% per treatment, so the outcome of this study can help prevent treatments that are unlikely to succeed. Additionally, if specific microbiota are related to the outcome, modulation might prove a viable tactic to improve the chances for successful IVF treatments. To obtain an answer to the research question of this chapter vaginal and urine samples were taken from a group of 297 women receiving IVF treatment, to subsequently perform a microbiota profiling. Our results showed that women with a low abundance of *Lactobacillus spp.* in their vaginal sample were less likely to have a successful embryo implantation. Surprisingly, and in contrast to the previous statement, an abundance of below 60% of *L. crispatus* was a predictor for becoming pregnant. These factors and various factors of smaller impact were worked into a predictive algorithm named the ReceptIVFity test. This test showed a predictive accuracy of 94% (sensitivity 26%, specificity 97%) in predicting a negative IVF outcome. This finding paves the way for potential interventions

in the future, however those would require further studies into this topic. For now this test can help couples make a better decision regarding the timing of their IVF treatment cycles.

Another point of interest that has become a point of debate recently is the existence of a urinary microbiota. Although the bladder is traditionally thought of as a sterile space, multiple studies have discussed finding microbiota closely resembling the vaginal microbiota through urine sampling. In **Chapter 7** an effort is made to clarify this topic. Samples and data from the women examined in the previous chapter were used for this. Vaginal and urinary microbiota found in these woman bore a striking resemblance. Notable deviations occurred as a large abundance of *E. coli* in the urine samples of some women, without showing up in the respective vaginal samples. These are likely due to subclinical cystitis in the urinary tract of the woman. We conclude from this, in combination with the relevant current literature, that there is no distinct urinary microbiota. Rather it is a dilution of the vaginal microbiota, possible acting as a reservoir for the vagina at times, which contaminates conventionally taken urine samples.

Concluding remarks

This thesis highlights the complexity of the interactions between pathogens, microbiota, and the host. The studies included in this thesis shed light on the interactions and prove the relevance of these interactions in the various infectious and reproductive processes. The findings in this thesis related to pathogen interactions with the host give clear insight into the pathogenesis of the pathogen. But perhaps more importantly, it sheds light on the biological background model of the clinical presentation in STIs. This is a topic that has consistently been linked to the presence and severity of later complications related to these infections. With the results of this thesis we hope to create a foundation not only for further research into these subjects, but also for translational efforts to put this knowledge to work in clinical settings.

However, this thesis also shows that there is still much work to do. In the case of infectious processes, the impact of microbiota on pathogens is often only described for the best studied pathogens. In the case of reproduction, current knowledge appears quite extensive, but significant pitfalls in study methodologies create a situation where even experts might have difficulty interpreting the information correctly. For all these current shortcomings though, these interactions are also fields where discoveries are happening at a lightning

fast pace. The potential impact of the implementation of vaginal microbiota diagnostics in healthcare, and especially in assisted reproductive therapies is already being noticed. To the point where complete adoption of the concepts is perhaps already inevitable. We recommend settings that plan to adopt the vaginal microbiota diagnostics to take extra care when researching the literature, but also to realize that benefits are likely to be great.