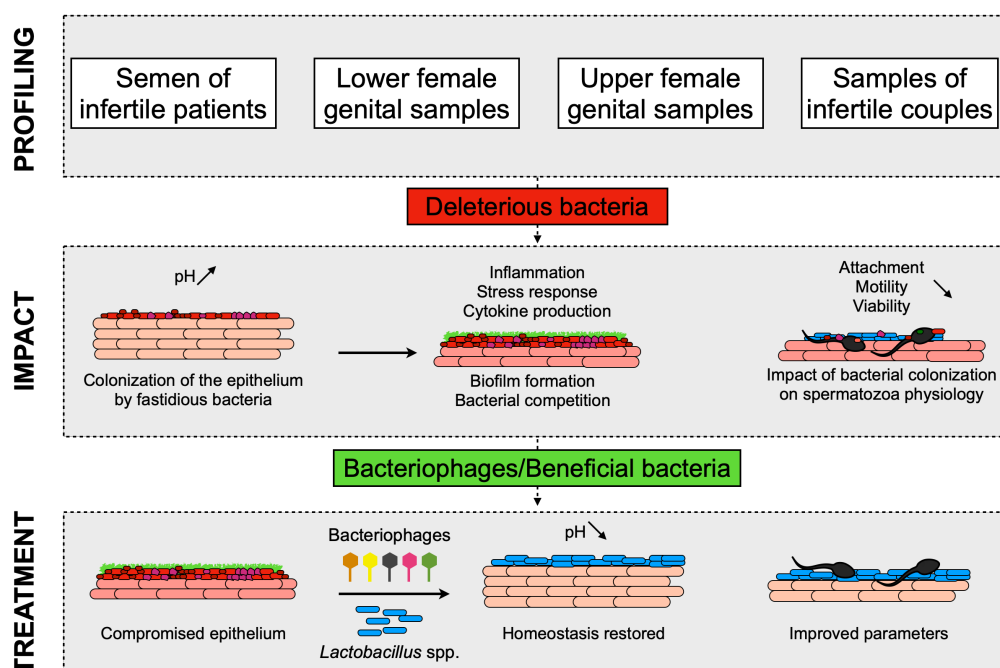


Role of genital microbiota in fertility and infertility – Prof Baud Lab

Bacteria and other microorganisms colonize most of the human body. They are not passive commensals but have a profound influence on the host homeostasis and play a significant role at multiple levels, including protection against pathogens, maturation of the immune system, metabolic pathways, vitamin synthesis and others. The human genital tract is not an exception to bacterial colonization. While the existence of a vaginal microbiota has been well established, other genital sites have been considered sterile environments with a general assumption that bacterial presence is a pathological situation. However, recent studies identified specific patterns of microbiota colonizing these sites, but their role remains poorly explored. Dysbiosis of the genital microbiota has been linked to the impairment of reproduction, including infertility, which affects one in seven couples. Unfortunately, male or female infertility is still considered “idiopathic” in a large proportion of cases. Studies on the impact of bacteria on human reproduction remain scarce and, when available, are descriptive analyses.

This ambitious PhD project has **three main goals**:

- Characterization of the microbiota from multiple genital sites and identification of beneficial/deleterious bacteria associated with each adverse outcome, with the establishment of a collection of genital isolates
- Evaluation of the impact of candidate bacteria/bacterial communities on spermatozoa and vaginal epithelium physiology
- Treatment for recovery of microbiota homeostasis using beneficial bacteria and/or phage therapy



Initially, will use targeted metagenomic approaches (16S rRNA profiling with Illumina sequencing) to identify bacterial species that have a potentially negative or positive impact on the studied body sites. In contrast to many metagenomic studies, our goal is not solely based on the description of microorganisms colonizing specific parts of the human body. We also want to understand how these bacteria interact with the host by using several methods. We will use reference strains, as well as isolates obtained from clinical samples, with the goal to create a large collection of genital isolates.

We have previously developed an *in vitro* model of infection of human sperm that allow us to monitor the impact of bacterial exposure on spermatozoal physiology. Using flow cytometry, we will monitor viability, mitochondrial membrane potential (directly linked with sperm motility), DNA fragmentation (TUNEL assay) and response to exposure to phosphatidylserine on the cell surface (Annexin V assay to follow apoptosis). The interaction will be further assessed using confocal microscopy and transmission electron microscopy.

To study vaginal colonization, our lab is currently developing an *in vitro* vaginal epithelium. This model is a promising alternative to the animal models, whose female genital physiology differs from that in humans, and will be used to study genital infections, dysbiosis and bacterial competition. The impact of the colonization on the epithelium will be assessed by lactate dehydrogenase (LDH) cytotoxicity assay and inflammatory responses. Flow cytometry using multiple markers will be used to characterize the impact of colonization on epithelial cells.

Once the effects of monobacterial cultures or complex communities will be established, we will explore the utilization of bacteriophages and beneficial bacteria as therapeutic strategies to eliminate fastidious bacteria and restore a normal physiological state, thus avoiding the broad use of antibiotics. We will screen filtrates coming from complex microbial communities to isolate specific bacteriophages, by using the double layer plaque assay. Once isolated, candidate phages will be characterized by electron microscopy and their genome will be sequenced. Activity of selected bacteriophages will be first evaluated in monobacterial cultures and subsequently in more complex bacterial communities, including biofilms.