Friends, enemies and everything in between

SUMMARY

This thesis reviewed the current knowledge about the role of the VMB in pregnant women from sub-Saharan Africa. It also investigated the VMB composition and the infection status of five burdensome STIs (*Chlamydia* (*C.*) *trachomatis*, *Neisseria* (*N.*) *gonorrhoeae, Trichomonas* (*T.*) *vaginalis, Mycoplasma* (*M.*) *genitalium*, human papillomaviruses (HPV)) among pregnant women living in Pemba Island, Tanzania. On this matter, this thesis provides the latest insights into the burden of vaginal dysbiosis, vaginal dysbiotic conditions, and STIs during pregnancy in sub-Saharan Africa and Pemba Island, in particular.

Pregnancy is a delicate period in a woman's life and pregnancy-related complications can be life-threatening for mothers and their children. Preterm birth, below average length for gestational age, low birthweight, pregnancy loss, and stillbirth are common pregnancy complications leading to morbidity and mortality among newborns. These complications are more common and more severe in middle and low-income countries, including sub-Saharan Africa. Several microorganisms ascending from the lower genital tract have been associated with adverse pregnancy outcomes. That is the case for diverse microorganisms (bacteria, fungi, viruses) able to colonize the vaginal environment. Such as the commensal vaginal microbiota (VMB) and microorganisms that can be transmitted after sexual contact, or sexually transmitted infections (STIs). Lactobacilli and other lactic acid-producing microorganisms in the VMB have been associated with a beneficial vaginal state. In contrast, an overgrowth of (facultative) anaerobes combined with a low abundance of *Lactobacillus* species (also known as vaginal dysbiosis) has been related to a less beneficial vaginal state.

Both vaginal dysbiosis, vaginal dysbiosis related conditions, and STIs such as *C. trachomatis*, *N. gonorrhoeae, T. vaginalis, M. genitalium*, HPV, herpes simplex viruses, and *Candida* species, promote a non-beneficial pro-inflammatory vaginal state and relate to various health conditions for expectant mothers and fetus. A common vaginal related condition that has been linked to maternal and neonatal morbidity is bacterial vaginosis (BV). Multiple studies of antenatal clinic attendees in sub-Saharan Africa observed that up to 40% of women had BV. Simultaneously, between 2010 - 2015, the pooled prevalence of *T. vaginalis, C. trachomatis, and N. gonorrhoeae* during pregnancy ranged between 4.6 - 31.4%, 5 - 9%, and 2 - 5.2%, respectively. Furthermore, during pregnancy, prevalence rates between 5.4 - 46% for HPV and 0.8 – 2.4% for *M. genitalium* have been reported in several independent studies from, and outside of, sub-Saharan Africa. Overall, it appears there is a high frequency of vaginal dysbiosis among women from sub-Saharan Africa or women with sub-Saharan ancestry. However, only limited data exists concerning the VMB composition during pregnancy, and new data for STI during pregnancy in rural settings of sub-Saharan Africa are still warranted. With this in mind, the burden of vaginal dysbiosis, VMB dysbiosis related conditions, and STI was investigated.

In the general introduction of this thesis, **chapter 1**, the VMB, its associated bacteria and conditions, along with the five STIs (*C. trachomatis*, *N. gonorrhoeae, T. vaginalis, M. genitalium*, and HPV) are described and introduced. The research setting is also outlined in **chapter 1**. The vaginal samples from pregnant and post-delivery women analyzed in this thesis were previously collected and stored in a biobank in Pemba Island, Tanzania. Pemba Island is one of the largest Islands in the Zanzibar archipelago, with approximately 19744 pregnant women, and 14000 annual births reported in 2018. Most inhabitants live below the UN poverty line, and the quality of antenatal and infant health care services remain poor. Limited data is available for STI burden among women from this Island, and to date, no information has been published on the VMB composition, particularly among pregnant women.

In **chapter 2**, the results of ten studies were systematically reviewed. These studies independently show that a *Lactobacillus*-dominant VMB or VMB containing Lactobacilli are the most prevalent, followed by a more diverse anaerobe-dominant VMB among pregnant women in sub-Saharan Africa. If further speciated, a VMB profile dominated by *L. iners* or *L. crispatus* were observed as the most common *Lactobacillus*-dominant VMB among pregnancy in several populations. Most pregnant women with an STIs (*C. trachomatis* and *T. vaginalis*) also had a *Lactobacillus*-dominant VMB, but with a significantly higher presence of anaerobic species in their VMB. For human immunodeficiency virus (HIV), another highly common STI in sub-Saharan Africa, one study reported an association between an anaerobic-dominant VMB, maternal HIV infection, and timing of antiretroviral therapy use before or during the pregnancy.

Moreover, use of probiotics (bacterial strains that can potentially modulate the microbiota) and other mineral or vitamin supplements with the VMB composition was also briefly addressed. Two of the included studies reported that the VMB did not differ between women who consumed probiotics and controls. Overall, the number of studies limited the evidence regarding the effect of STIs, medication, and supplements on the VMB. Thus, the use and efficacy of *Lactobacillus*-based probiotics, medicine (antiretroviral therapy), and other supplements on the VMB among sub-Saharan pregnant women need to be further investigated.

Previous studies have linked VMB dysbiosis and dysbiotic conditions with various adverse pregnancy outcomes among different populations. To gain more insights into this association among sub-Saharan African women, **chapter 3** systematically reviewed the findings of twelve studies that investigated this subject. However, merely one culture-based study from 1993 reported about the relationship between VMB related bacteria and pregnancy outcomes. They observed that pregnant women in sub-Saharan Africa with VMB without Lactobacilli, or with overgrowth of other bacteria at the first antenatal examination, had 3.6 times higher risk of giving birth to a newborn with low birth weight (< 2 kg). This was in comparison women with a *Lactobacillus*-dominated VMB or mixed bacteria with a low abundance of *Lactobacillus* species. More studies were retrieved investigating the association between BV and several

adverse pregnancy outcomes among women from sub-Saharan Africa. An association between BV and preterm birth was most often reported (7/9 studies). None of the studies included found an association between BV and pregnancy loss (5/5) or intra-uterine growth retardation (1/1). Finally, there were discrepancies or a low number of studies to support the evidence between BV and low birthweight (2/6), PROM (2/4), intra-uterine infections (1/1), and small for gestational age (1/1). For another but a less well-known vaginal dysbiotic condition, aerobic vaginitis (AV), no article was retrieved that investigated its role with pregnancy outcomes in sub-Saharan African populations. Due to the considerable differences in study design and outcome reporting, the high burden of BV in sub-Saharan Africa, and the limited studies available on VMB composition and AV, additional research is needed to better determine the role of VMB dysbiosis, BV, and AV among sub-Saharan African women.

As highlighted earlier some sexually transmitted microorganisms can cause pregnancy-related sequelae. Thus, the burden of infections should continuously be monitored in vulnerable populations, such as pregnant women. In **chapter 4**, the burden of four non-viral STIs was investigated in vaginal samples from Muslim-Shirazi women between 16-48 years old in Pemba Island, Tanzania. In 55 vaginal samples from 439 (12.5%) local pregnant women, at least one STIs was detected. The prevalence of *T. vaginalis* (7.1%) was the highest, followed by *C. trachomatis* (4.6%) and *M. genitalium* (2.1%). The presence of *N. gonorrhoeae* was not detected.

In chapter 5 the (natural) course of the four genital pathogens in pregnant women from Pemba Island were investigated. When compared to chapter 4, more vaginal samples across two timepoints (before 20 gestational (GA) weeks and after 20 GA weeks) during pregnancy and one timepoint post-delivery (42-62 days after parturition) were investigated for the presence and persistence of STIs across timepoints. *N. gonorrhoeae* was detected in vaginal samples from two different women. This further analysis provided evidence of this pathogen's presence in Pemba Island's local population. Interestingly, during pregnancy, the combined prevalence of STI was higher at the second timepoint (n = 385; 16.7%) compared to the prevalence at the first timepoint (n = 257, 12.2%). Moreover, in 44 post-delivery samples, the prevalence of *T. vaginalis* (6.8%) was the highest, followed by *C. trachomatis* (2.3%), and *N. gonorrhoeae* (2.3%), while *M. genitalium* was not detected at this timepoint. Owing to the low prevalence of *N. gonorrhoeae*, its natural history could not be determined. Some women cleared off *T. vaginalis* (n = 11; 18%) and *M. genitalium* (n = 4; 25%) infections within ten weeks following initial detection during pregnancy. Clearance post-delivery was observed for *T. vaginalis* (n = 5; 80%) approximately 22 weeks after the last detection in pregnancy. Persistence for *C. trachomatis* during (n = 11) or after pregnancy (n = 1) was observed in all of the vaginal samples from the infected women. The study cohort size was small; therefore, careful extrapolation of results is recommended. However, the findings **in chapter 5** support the need for further investigation in a bigger cohort, and the usage of biobanked samples is a valuable approach in this sense.

The burden of 15 high-risk HPV (hrHPV) genotypes among local pregnant women in Pemba were further investigated in **Chapter 6**. The prevalence of high-risk HPV was between 5.9% and 11.2% in pregnant women in Pemba Island, Tanzania. The persistence rate for high-risk HPV during pregnancy was high (63.6%) in this Tanzanian cohort. For future implementation of this assay in resource-poor setting, it is worth mentioning that the HPV detection kit used in **Chapter 6** does not require additional DNA extraction or purification. Additionally, it is based on an isothermal amplification system to amplify targeted sequences in the different HPV genotype regions. This is an example of a simple sample processing procedure method that might be helpful for HPV screening in clinical diagnostic settings, especially in resource-limited laboratories.

The findings in **chapter 7** show for the first time that among the tested pregnant women from Pemba Island, the VMB was generally *Lactobacillus* dominant during pregnancy (65% before 20 GA weeks and 81% after 20 GA weeks) and non-*Lactobacillus* dominant (74%) postdelivery. During pregnancy, the most common *Lactobacillus* species were *L. crispatus* at the first timepoint (26.5%; the prevalence was 1.5% higher than *L. iners*) and *L. iners* at the second timepoint (42.5%). VMB richness (number of species) significantly decreased during pregnancy, while the VMB diversity (number of species and their relative abundance, assessed by the Shannon diversity index) significantly increased between the second pregnancy timepoint and post-delivery. Data from matched samples show that in almost 50% of the women (n = 38) tested at both timepoints the VMB shifted profile during pregnancy. This shift was most commonly towards an *L. iners* dominant VMB. From the second pregnancy to post-pregnancy timepoints, a switch to a diverse VMB community accounted for 85% of these changes. Several pathobionts (VMB related bacteria with potential pathological sequelae) were identified in the tested samples, with *Klebsiella species* and *Streptococcus anginosus* as the most prevalent during and after pregnancy. The substantial presence of pathobionts was most commonly detected in diverse, non-*Lactobacillus* dominant VMB, followed by *L. iners* dominant VMB. There was a higher frequency of pathobionts and a substantial presence (higher relative abundance) of pathobionts in vaginal samples collected post-delivery compared to samples collected during pregnancy. At the second timepoint during pregnancy, significantly more women carrying a genital pathogen (HPV, *C. trachomatis*, *T. vaginalis*, and *M. genitalium)* had VMB belonging to by *L. iners* dominant VMB. Evidence from this study enhances the understanding of VMB during pregnancy and in the post-delivery period in a (rural) sub-Saharan African population.

Based on the evidence of the vaginal environment *friends* (lactic-acid producing microbes, such as most species belonging to *Lactobacillus* genus and particularly *L. crispatus*), *enemies* (STIs), and *frenemies or everything in between* (e.g., pathobionts) during pregnancy and after pregnancy, a general discussion and recommendation of this thesis are provided in **chapter 8**.

A high frequency of non-*Lactobacillus* dominant VMB is reported among pregnant women living in sub-Saharan Africa, including Pemba Island, Tanzania. Furthermore, among Pemban women, the burden of viral, bacterial and protozoan STIs were also revealed during and after pregnancy. Post-pregnancy, most women in Pemba had a diverse, non-*Lactobacillus* dominant, VMB profile. Various hosts (including host-genetics, hormonal and inflammatory response), microbial, behavioural, sociodemographic, nutritional, and environmental factors directly or indirectly influence VMB composition and susceptibility to STIs in pregnancy. However, microbiology methods to detect microorganisms in the vaginal space, and a consensus approach to characterize the VMB profiles, should be further developed to provide more and improved evidence-based VMB and STIs related research, including for resources-limited settings. An integrated approach involving different stakeholders (researchers, clinicians, and policymakers) should be considered to implement better research, policy and management strategies. These efforts are crucial to improve female and newborn health globally, particularly in sub-Saharan Africa, with implications for society and future generations.