

Reproductive Tract Microbiome in Animals: Physiological versus Pathological Condition

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15.1 Introduction

The reproductive system consists of external genitalia as well as internal genitalia. The study of microbial community inhabiting reproductive tracts of animals is essential to understand reproductive physiology and health aiming not only towards a clinical cure but also to restore healthy microbiota status. The “one health” concept focuses on the ecological relationships between animals, humans, and environmental health (Davis et al. 2017). Hence, a better understanding of the microbiome in humans, animals, the shared environment, and their interactions could help to prevent diseases (e.g., occupational) and manage human health or various disease states.

Presently, the “sterile womb” concept, where a fetus grows up in a microbial-free (sterile environment), is under argument (Perez-Munoz et al. 2017). Recent studies have indicated that the cervical mucus plug may not be completely impermeable to ascending microbes from the vagina and may allow the transport of bacteria into the intrauterine cavity (Hansen et al. 2013; Baker et al. 2018). The reviews have been focused on correlations between commensal uterine microbial species and fertility problems, including pregnancy complications in humans (Franasiak and Scott 2017; Moreno and Franasiak 2017). Host–microbiota depends on their symbiotic relationship, thus acting as a natural barrier against colonization by pathogenic species. Hence, homeostasis is constructed and maintained. In animals particularly, determining the host–microbiome relationship within the reproductive tract may help to devise better

tools for enhancing reproductive efficiency, such as treatment with probiotics to introduce microbial communities that result in positive results with regard to reproduction (Clemmons et al. 2017).

15.2 Vaginal and Uterine Microbiome in Animals

The common phyla found between the uterus and the vagina are most commonly found in many host–microbiome relationships in many species (Jami and Mizrahi 2012; Myer et al. 2015), and their function is not yet fully understood. However, relative abundance of these phyla changes according to the changes in the host physiology. The human vagina with stable bacterial communities has been related to a healthy immune state of the female reproductive tract (Gajer et al. 2012; The Human Microbiome Project Consortium et al. 2012; DiGiulio et al. 2015). Recently, the use of sequencing technologies has gained importance to characterize the microbiome. Some shared operational taxonomic units (OTUs) show there is an interaction between bacterial communities of uterus and vagina, yet the OTU differences between the vagina and uterus are due to functional differences in the tissue and microbial ecosystem niche (Clemmons et al. 2017). Both vaginal and uterine microbiota are less explored in animals, and the microbiota is composed of anaerobic, facultative anaerobic, and aerobic microorganisms (Otero et al. 2000). In postpartum

cows, estrus synchronization followed by vaginal and uterine bacterial population study before insemination showed Firmicutes as the most abundant phyla in uterus and vagina (Clemmons et al. 2017), also between resulting pregnant and nonpregnant cow's uterus and vagina (Ault et al. 2019) (Table 15.1). The uterus samples of the virgin and pregnant heifers showed Firmicutes, Bacteroidetes, and Proteobacteria phyla in abundance, and this study indicates the presence of common phyla of the gastrointestinal tract inhabiting the reproductive tract (Moore et al. 2017). Microbial communities present in the uterus are also found in the vagina and gut; therefore, microbiota from the vagina and cervix migrate via intra-uterine ascension, where they will subsequently colonize the uterus (Moore et al. 2017). The possible blood-borne transmission of the gut microbiome to the uterus in cows has been explored (Jeon et al. 2017). The vaginal microbiome is dominated by Proteobacteria, Bacteroidetes, and Actinobacteria in the mares. Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria are abundant in the equine endometrial microbiome (Swartz et al. 2014). Mare endometrium during estrus showed only proteobacteria-driven microbiome (Heil et al. 2018); the estrous cycle likely influences the changes in the microbiome. During estrus, dynamic changes of the mare cervix influence communication between the vagina and the uterus. Ewe microbiota exhibited greater diversity compared to the cow with Bacteroidetes, Fusobacteria, and Proteobacteria being the dominant phyla. Archaea and lactobacilli were prevalent, but not abundant (Swartz et al. 2014) (Table 15.1). Vaginal microbiome in bitches is dominated by Bacteroidetes, Proteobacteria, Tenericutes, and Firmicutes phyla, while Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes were the most prevalent phyla in the uterus similar to a cow (Table 15.1). At the genus level, the vaginal bacterial community of bitches was higher in richness and the uterus was higher in diversity (DeSilva et al. 2019). Similar to mares, the stages of estrous cycle influence in determining diversity and richness of microbiome in uterus and vagina in bitches.

The dominating vaginal microbiomes in the most wild primates are Firmicutes phylum, followed by Fusobacteria, Bacteroidetes, Proteobacteria, and Actinobacteria. Interestingly, the vaginal microbiome of the wild chimpanzee (the closest human relative) was dominated by Fusobacteria compared to Firmicutes and had <3.5% Lactobacilli (Yildirim et al. 2014). This is in contrast to the captured baboon, where the dominant phylum was Firmicutes but mainly consists of Clostridia genera (Uchihashi et al. 2015). The samples from wild howler, red colobus monkeys, and lemurs showed few or no reads for Lactobacilli species. The lemurs' vaginal microbiome especially showed a significantly higher proportion of unclassified taxa, suggesting possibly novel bacteria taxa not previously characterized (Yildirim et al. 2014). The Firmicutes were also the dominant phyla in guinea pig, and *Lactobacillus* spp. had a very low relative abundance with other microbes from *Corynebacterium*, *Anaerococcus*, *Peptoniphilus*, *Aerococcus*, *Facklamia*, and *Allobaculum* genera being more dominant (Neuendorf et al. 2015). Similar to mares, the semi-captive giant panda vaginal tract had Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes as the most abundant phyla and Proteobacteria followed by Bacteroidetes

in the uterus (Xia et al. 2017). The human vagina is dominated by commensal bacteria, predominantly Lactobacilli; therefore, this vaginal microbiota has an established role in female reproductive physiology, pathogen defense, and function (Wee et al. 2018). However, the Lactobacillus abundance in the reproductive tract of bovine is very low (Swartz et al. 2014; Clemmons et al. 2017). However, the reproductive tract microbiome of bovine is significantly more diverse than that of humans. The similarities exist between the uterus and the vagina of humans and bovine, where Firmicutes dominates both (Laguardia-Nascimento et al. 2015; Clemmons et al. 2017). The bacterial communities have been widely studied with less emphasis on the Archaea and Fungal communities in the vagina and uterus in animals. The vaginal, uterine, and placental microbiomes of humans and many other species have been sequenced, including the vaginal microbiomes of wild primates (chimpanzee, baboon, howler, red colobus monkey, and lemur), the guinea pig, cow, ewe, bitch, mare, and giant panda (*Ailuropoda melanoleuca*) (Figure 15.1).

15.3 Factors Affecting Reproductive Tract Microbiome

- a. *The cervicovaginal microbiome:* The vaginal microbiome fluctuates according to different physiological conditions such as puberty, menstruation, sexual activity, and pregnancy (Giudice 2016; Prince et al. 2015; Song et al. 2017). In humans, the onset of puberty brings the vaginal flora under the influence of reproductive hormones, and environmental pH decreases causing predominance of *Lactobacilli* spp. (Moreno and Franasiak 2017). The microbiota development and the relative abundance of OTU greater in the vagina tract than in the uterus are strongly influenced by the proximity of the gastrointestinal tract (Clemmons et al. 2017 and Laguardia-Nascimento et al. 2015). The vaginal microbiome participates simultaneously in the host's metabolic and immune systems, thus maintaining homeostasis and contributing significantly to the reproductive success and prevention of disease (Clemmons et al. 2017; Giudice 2016).
- b. *The uterine microbiome:* The comparison between uterine and vaginal microbiome showed differences between species. In healthy cows, increased richness and greater levels of diversity were found in the vagina in comparison with the uterus, which is possibly due to proximity to the external environment (Clemmons et al. 2017). In healthy women, the uterine endometrial microbiome showed lower relative abundance compared to the cervix microbiome (Verstraelen et al. 2016; Wee et al. 2018), whereas in mares, the relative abundance of the external cervical os is similar to the uterine relative abundance. The differences in the anatomy of the reproductive tract suggest the changes in the microbial communities in each species. Invasion of microbes into the