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Relationship between diversity of the genital tract microbiome and cattle fertility

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Abstract

Numerous microorganisms are present in the female cow's reproductive system from an early age. Other dangerous microorganisms can occasionally enter the genital canal and result in illnesses that reduce fertility. Normally, commensal microorganisms support genital tract homeostasis and generate substances that arouse male sex. Typically, the vaginal microbiota of healthy women has low diversity in relation to This diversity is in line with the low pH (4.5) that is maintained by the various prominent *Lactobacillus* species' production of lactic acid, which is thought to limit the colonization of vaginal infections. Not all *Lactobacillus*, meanwhile, are thought to be similarly advantageous. Even before calving, the vaginal microbiome of cattle is already developed. Unavoidable microbial inflow into the reproductive system of cows during calving or mating is likely to change the composition of the commensal microflora. Uterine bacterial microbiota in healthy and metritic postpartum dairy cows: a metagenomic investigation. These bacteria are crucial for immunomodulation, pathogen defence, and maintaining the vaginal mucosal barrier's structural integrity. Furthermore, a male and a female's semichemical communication is facilitated by the genital microbiota. To support the health of the female vaginal tract, the microbiome must have an ideal balance between its variety and abundance. Dysbiosis, genital infections, and disturbed fertility result from the disruption of this equilibrium. Animal antibiotic usage must be decreased as part of the worldwide One World, One Health initiative. This field of study has the potential to add to our understanding of the relationship between the endometrial microbiome and fertility, particularly how it functions probiotically in various species.

Keywords: Genital organ, microbiology, cattle species, pathogens

Introduction

The phrase "microbiota" describes all categories of bacteria, viruses, parasites, and fungi that are present in a specific place or habitat. [Human Microbiome Project Consortium, 2012] ^[1]. Within 20 minutes of calving, cows generate a specific endometrial microbiome that remains constant until at least the second postpartum day in both cows that develop metritis and those that do not. Even before calving, animals have microbes living in the uterus. In terms of relative abundance, Bacteroidetes and Fusobacteria are more common in cows with metritis than Proteobacteria and Tenericutes [Jeon, 2015] ^[2]. Additionally, the vaginal tracts of various species, including cattle, include a variety of bacteria, protozoa, fungus, and viruses [Lacroix, 2020] ^[3]. While *T. pyogenes*, *Fusobacterium necrophorum*, *Bacteroides* spp., and *Prevotella* spp. are among the mixed bacteria that typically cause uterine infection (Sheldon, Dobson, 2004) ^[4]. The genital tract contains a variety of microbial species, including *Lactobacillus* and *Bacteroides*, depending on the organ. Only a few research on vaginal microbiota have mentioned women from urban or rural locations in underdeveloped nations, while the majority have concentrated on urban women in industrialized countries. Asian migrants who arrived in the Americas 14,000–24,000 years ago gave rise to the Amerindian peoples, who have continued to live in isolation and undergone genetic divergence. The colonial era saw the entrance of Europeans and Africans, which caused admixture 43 and gave rise to the current mestizo population that predominates in South America. There are still remote Amazonian settlements where people continue to live pre-agricultural lifestyles with no access to healthcare, no access to electricity or running water, no market economy, and a reliance on fishing, hunting, and farming methods. Migration to places with better access to jobs, education, and healthcare services. The vaginal tract microbiota has attracted a lot of attention from scientists in recent years, to the point that it is now believed to be related to uterine disorders or infertility. Through various microbial competition, the vaginal microbiome plays crucial roles in the female reproductive system.

Symbiotic bacteria produce a biofilm that works in conjunction with the cervicovaginal mucus to guard the genital canal from disease invasion [Tang, 2008] ^[9]. Additionally, commensal microorganisms create beneficial chemicals including reactive oxygen species and lactic acid that prevent the spread of diseases [Tachedjian, 2017] ^[11]. Some *Lactobacilli* species have been linked to both normal delivery and protection of the fetus during pregnancy [Romero, 2019] ^[12]. Other areas of cattle reproductive function affected by the vaginal microbiome, such as pheromone generation and semi-chemical signaling, were the subject of some recent research [Srinivasan *et al.*, 2021] ^[13]. While the vagina is thought to be the primary source of endometrial microfauna, especially during the times when the cervical lumen is less constrained at estrus, breeding, or parturition, whereas the genital microbes are thought to originate from the environment or different organs such as the rumen, skin, rectum, or feces. During these times, vaginal mucous is less thick, allowing it to flow. It appears that a variety of variables, some of which are unique to females including estrous cyclicity and pregnancy, have an impact on the richness of the vaginal microbiome. The importance of extrinsic variables including vaginal diseases and diet cannot be overstated, though. The majority of genital tract bacteria are non-pathogenic, and they live in harmony with enterocytes here. Dysbiosis, infertility or genital illnesses such as vulvovaginal candidiasis can occur in humans when the body's natural microbial diversity is altered. The vaginal microbiome of cattle was the topic of this review research, which also examined how it compared to the human microbiome, which has been widely studied in relation to genital infections and infertility. It was helpful to include findings from several articles in this review with the most pertinent discoveries that can be utilized in the animal area because the human microbiome has been studied more thoroughly than other species. In this review, we concentrate on the origin, diversity, and clinical importance of microbiota as well as the likelihood of cattle developing uterine illness or infertility. We also look at how this knowledge might be used to guide the development of probiotic and prebiotic treatments for uterine illnesses.

The most prevalent phyla in the vaginal tract of cattle and sheep are the Bacteroidetes, Fusobacteria, and Proteobacteria groups. The most prevalent genera at the species level were *Aggregatibacter* spp. and *Streptobacillus* spp. Intriguingly, vaginal samples from sheep and cows both contained 80% and 90% of the *Lactobacillus* spp., the predominant microbial communities in the human genital system.

The conventional wisdom holds that the endometrial environment is sterile, especially while a woman is pregnant. It is now understood that bacteria in the calving region might enter the cow's uterus during calving. In laboratories, culture-based microbiological tests are frequently used to identify the bacterial species that are causing the invasion [Karstrup, 2017] ^[26]. The uterus of cattle has a dynamic microbiota, according to findings when utilising independent culture techniques of sequencing. The ground breaking discovery that the style of delivery determines which varied bacterial species to which human neonate is exposed to led to new discoveries in the field of animal behaviour (Dominguez-Bello *et al.*, 2010) ^[28].

Importance of microbiome of genitalia

In terms of the precise taxonomic categorization and

functional aspects of the microbiome, which are useful for the development of diagnostic techniques such as microbial biomarkers and dysbiosis indices, the reproductive tract microbiome of cattle is relatively under-explored. In both humans and animals, the genital microorganisms act as key pathogen defenses. *Lactobacilli* in women create lactic acid, which controls the pH of the vagina and prevents the spread of diseases. Additionally, lactic acid causes the vaginal environment to become more acidic, interfering with intracellular processes and causing the removal of bacteria. [Alakomi, 2000] ^[31]. *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Escherichia coli* (*E. coli*) are all inactivated when there are normal amounts of lactic acid, according to the findings of *in vitro* research [Nardini, 2016] ^[32] and [Messman, 2020] ^[47]. In the study by Deng *et al.*, 2019 ^[17], Firmicutes (31.57%), Proteobacteria (24.08%), Bacteroidetes (12.96%), and Tenericutes (4.95%) were the most prevalent vaginal microbes. These two recent research' findings show that there are considerable differences in the proportions of the dominating microbial communities between people. The prevention of human immunodeficiency virus type 1 (HIV-1) is one of the possible advantages of the commensal bacteria in women. It appears that vaginal *Lactobacilli* shield women from acquiring HIV-1 while sexual intercourse. The manner of birth and surroundings have an impact on the microbiota makeup in newborns. In humans, the general microbiome of the newborn delivered vaginally and the mother's vaginal microbiota, which is dominated by *Lactobacillus*, *Prevotella*, or *Sneathia* spp. Caesarean-section babies' general microbiomes are primarily composed of *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* species and are comparable to the skin microbiomes of their mothers. The two most common species at the genus level were *Aggregatibacter* spp. and *Streptobacillus* spp. While *Lactobacilli* are found in 80% of sheep and 90% of cows, the whole microbial community is less plentiful, creating a near-neutral vaginal environment as opposed to an acidic one in women, where *Lactobacilli* are found in greater numbers. Cows and ewes also share numerous species, primarily *Sneathia* spp., *Porphyromonas* spp., and *Prevotella* spp., in addition to *Lactobacilli*. A low Firmicutes to Bacteroidetes ratio is a precursor to the development of postpartum endometritis in cows. Lactic acid and the resultant biofilm reduce the amount of free viruses present, reducing the likelihood of HIV being shed.

Genital Microbiome Microbiota

Vaginal diversity

There are 15 taxa in the vagina of cattle having no uterine infections, majority being the *Bacteroides* (28.3%) and Enterobacteriaceae (17.8%), as well as *Victivallis* (7.2%), *Streptococcus* (6.1%), *Phyromonadaceae* (5%), *Alistipes* (3.9%), *Coriobacteraceae* (3.3%), *Clostridium* (3.3%), *Betaproteobacteria* (2.8%). Cows with reproductive diseases such as purulent vaginal discharge have a more diverse vaginal microbiome containing 68 taxa, dominated by *Bacteroides* (35.83%), Enterobacteriaceae (18.62%), *Histophilus* (8.79%), *Alistipes* (4.34%), *Flavobacteriaceae* (1.77%), *Victivallis* (8.49%), *Coriobacteriaceae* (2.44%), *Streptococcus* (2.09%), *Barnesiella* (2.03%), and *Oscillibacter* (1.24%) [Deng *et al.*, 2019] ^[17].

Cervicovaginal microbiota

At the cervicovaginal and introital vaginal sites, samples were

collected by a doctor and immediately frozen for later analysis. A DNA sample was taken, and the 16S rRNA region V1–V3 was amplified and sequenced. 222 cervicovaginal and introital samples from 111 women yielded a total of 2,771,167 sequences (13, 196 sequences/sample); eight environmental controls only produced 152 sequences. The pattern in the environmental controls and the low sequence number imply that there was little to no impact of contamination on the makeup of the human samples. The rarefying process at 1,655 sequences/sample resulted in the loss of all environmental control samples. Also lost were 25 (11%) samples with a low sequence number. 196 samples in total—95 cervicovaginal and 101 introital—were studied. All ASVs identified as *L. iners* underwent a separate analysis. 43 of the 90 *L. iners* ASVs in total were found in several women. Sixty-six percent of women had one of the three most common *L. iners* ASVs, five percent did, and two percent had the others. These three *L. iners* ASVs showed no correlation with urbanization, ethnicity, or HPV infection.

Uterus

It is now understood that cows' uteri naturally contain a microbiome during gestation. While the uterine microbiome is less diverse than the vaginal microbiome, it does derive mostly from the vagina and, to a lesser extent, from the skin and stomach. In the uterus, bacteria are constantly present. *Porphyromonas levii*, *T. pyogenes*, and *F. necrophorum* were found in pregnant cows. It's interesting to note that opportunistic bacteria like Mycoplasmataceae and *Histophilus* can develop into pathogens. Furthermore, the presence of an abundance of bacteria in the uterus prior to calving is not accompanied with inflammation, suggesting a higher level of microbial tolerance during gestation. *E. coli* and *A. pyogenes* are important players in the development of metritis, according to specialised literature.

Over a woman's lifespan, her genital microbiota evolves. Animal reproductive tract microbial communities are spontaneously chosen due to their symbiotic roles. For instance, *Lactobacilli* cling to the vaginal mucosa in women by using their tiny membrane extensions, called fimbriae. The collagen-rich vaginal tissue is also an important source of nutrition for *Aggregatibacter* spp. In addition, there are additional variables that influence the variety of the genital microbes, some of which are intrinsic, like diet, and others which are extrinsic, like the stage of the female reproductive cycle. Given the striking similarities in the microbial populations of the two anatomical regions, it is intriguing to speculate that the vaginal microbiome may have evolved from the gut microbiota.

Intrinsic Factors

Bovine vaginal tract microbial species are thought to vary among individuals, which may have an impact on reproductive outcomes. This difference could help to explain why some animals get resistant while others contract uterine illnesses. Other mammals, including humans, are thought to exhibit these variances frequently.

Species

The diversity of the vaginal microbiome among animal species and also within an individual animal disturbs the control of reproductive hormones. For instance, estradiol concentrations were lower and the duration to ovulation during the follicular phase of the reproductive cycle was

considerably delayed in two different investigations utilizing either cows with endometritis or those administered bacterial lipopolysaccharide (LPS). The vaginal microbiomes of cattle were composed of a large abundance of *Enterococcus* spp., *Staphylococcus* spp., and *Streptococcus* spp., which was different from the vaginal microbiome of ewes where there was a predominance of *Bacillus* spp., *Corynebacterium* spp., *Escherichia* spp., *Staphylococcus* spp., and *Streptococcus* spp. At the phylum level, Proteobacteria, Fusobacteria, and Bacteroidetes dominated the vaginal microbiomes of both cows and ewes.

Breed

The vaginal microbiome of Gyr cattle, a popular dairy breed in South American nations like Brazil, is loaded with bacteria and fungus, with just a modest population of archaea. [Giannattasio-Ferraz, 2019] ^[49]. The most often found bacteria were Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria. The two fungal genera that were found the most commonly were *Mycosphaerella* and *Cladosporium*. The *Methanobrevibacter* genus was the most prevalent whereas archaea were scarce. The vaginal microbiome of Nellore beef cattle is primarily made up of Firmicutes, Bacteroidetes, Proteobacteria, and up to 20% of unclassified bacteria. The leading archaeal genus was *Methanobrevibacter*, while the most prevalent fungus genus was *Mycosphaerella*. The vaginal microbiome of Holstein Friesian cow, the most common dairy breed in North Africa, Europe, and the USA, was primarily made up of bacteria from the Firmicutes, Tenericutes, Proteobacteria, and Bacteroidetes groups. Actinobacteria and Spirochaetae were among the other bacteria that were discovered in lesser concentrations. [Quadros *et al.*, 2020] ^[48].

Pregnancy

The vaginal microbiome's microbial diversity decreases and its archaeal population increases throughout the gestational period. Due to the significantly greater progesterone concentrations that cause the vaginal microbial community to be suppressed, the species of bacteria present in the vagina during the gestational period are less diverse. Similar to animals, humans also experience a decline in vaginal microbial population as gestational period lengthens. Contrary to humans, cattle have rather constant vaginal microbiomes during pregnancy. *Pasteurellaceae* species and *Fusobacterium* species were prevalent in the vagina of non-pregnant heifers, however *Pasteurella multocida* was found frequently in pregnant heifers.

Postpartum

The uterine microbiota of cows without uterine infections during the first month postpartum showed significant individual variation. [Sheldon, 2008] ^[25]. The bacterial diversity in the uterus of cows with and without uterine inflammation was similar at 10, 21, and 35 days postpartum (DPP), but the uterine microbiome was noticeably different between the two groups. Alpha and beta diversities were unaffected by the postpartum day of sampling. Based on findings from a metagenomic investigation of uterine samples taken three times during the first 35 DPP, *Porphyromonas*, *Bacillus*, *Schlegelella*, *Paracoccus*, and *Fusobacterium* made up the majority of the uterine microbiome of cows without uterine inflammation.

Conclusions

The genital microbiota represents an opportunistic field of study in the realm of cattle fertility when considering the microbiome is an essential immunological barrier against pathogens and for pheromone production. Specific questions that can be addressed are the functions of specific bacteria taxa involved in physiological uterine involution in postpartum cows. Certain bacterial profiles have implications on the temporal stability of the vaginal microbiota. Ecologically, shifts from *L. iners*-dominated to non-*Lactobacillus* dominated profiles are more frequent than shifts to any other *Lactobacillus*-dominated profile. *L. iners* appears to be better suited to environmental changes, such as hormone levels, based on genomic and metabolic traits. Due to high pregnancy rates (mean of 4.4 pregnancies/woman in this study), *L. iners* dominance would be advantageous for maintaining a *Lactobacillus*-dominated profile in women with frequent hormonal shifts. Given the Asian origin, one should anticipate genetic similarities between Amerindians and Asians. In fact, several investigations have revealed a high incidence of *L. iners*-dominated vaginal profiles in South Asian Surinamese women and Asians living in the USA. Modulating the vaginal microbiome may be a useful alternative to antibiotic therapy since dysbiosis can lead to uterine illness. As a result, probiotic management of genital postpartum uterine infections may be achieved by intravaginal inoculating cattle at risk of infection. Although the uterine microbial composition has been thoroughly researched using conventional culture, no approaches that are not culture-dependent have yet been tested. In this work, we examine if significant differences exist between puerperal metritic and healthy cows in terms of the bacterial assemblage of the intrauterine environment of postpartum dairy cows.

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