



The Reproductive Microbiome: A Hidden Regulator of Fertility in Domestic Animals

Lakshmi Yadav^{1*}, Neeraj Srivastava^{1*}, Dhaval J Kamothi², Usha Yadav¹, Shubhangi Prakash Suryawanshi¹, Amala Jackson¹, Prathamesh Mergewar¹, Pratyanshu Srivastava¹ and Amrita Priyadarshini¹

¹Division of Animal Reproduction, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, Uttar Pradesh, India.

²Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Bhuj, Gujarat, India.

* Corresponding author. E-mail: Ydvlakshu01@gmail.com, sangnee15@gmail.com

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ABSTRACT

Researchers have long attributed the reproductive capability of domesticated animals primarily to genetics, hormonal interplay, nutrition, and animal management. Now, the new breakthroughs in high-throughput sequencing technologies have identified another important factor - the reproductive microbiome. In particular, it was revealed that the microbial communities of both the male and the female reproductive tracts have active interfaces with host immunity, hormone signalling, gametes, and embryos. The seminal microbiome of bulls, boars, stallions, and bucks has been linked to sperm motility, morphology, and fertilizing capacity, and, because artificial insemination deposits semen-borne bacteria into the female tract, seminal and female-tract communities can interact to influence conception outcomes. The new data received from different livestock species show that the maintenance of normal microbiota is important for uterine health, semen quality, fertilization, implantation, and the maintenance of pregnancy, whereas changes in the microbiome composition are characteristic of infertility, post-partum uterine disease, repeat breeding syndrome, and early loss of embryos. However, most of this evidence remains associative: confounding by parity, metabolic status, hygiene, and antibiotic use, together with contamination of low-biomass samples, means that causal links between specific taxa and fertility are still largely unproven. Although many questions are still unclear, the development of microbiome studies is changing reproductive biology rapidly and opening new perspectives for precision veterinary medicine. The current article is devoted to a review of recent advances in microbiome research in reproduction and the discussion of their applications in practice by veterinarians and livestock producers.

INTRODUCTION

Reproductive efficiency is a foundational pillar of sustainable livestock production, directly dictating herd longevity, genetic progress, and enterprise profitability. Despite notable advancements in nutritional management, cryopreservation protocols, assisted reproductive technologies (ART), and hormonal synchroniza-

tion regimens, reproductive failure remains the primary cause of involuntary culling across dairy herds and livestock enterprises worldwide (Forcina et al., 2022). Subfertility manifests as protracted calving-to-conception intervals, elevated services per conception, repeat breeding syndrome, and early embryonic loss, incurring billions of dollars in aggregate annual losses across the livestock sector. Historically, reproductive success was viewed almost exclusively

through the lenses of gametogenesis, endocrine signalling, nutritional status, and biosecurity (Srivastava et al., 2013; Ponraj et al., 2016). However, modern culture-independent high-throughput sequencing has revealed a neglected biological determinant: the reproductive tract microbiome. In microbiome research, precise terminology is vital: “microbiota” refers specifically to the taxonomic assemblage of living microorganisms (bacteria, archaea, fungi, and viruses) inhabiting a defined anatomical niche, whereas “microbiome” encompasses the entire ecosystem, including microbial collective genomes, structural elements, metabolites, and surrounding environmental conditions.

Targeted 16S rRNA gene amplicon sequencing and shotgun metagenomics have revolutionized reproductive immunology and theriogenology, demonstrating that reproductive mucosal surfaces harbour dynamic microbial ecosystems that actively modulate mucosal epithelial integrity, gamete survival, steroidogenesis, and embryonic receptivity (Chen et al., 2017; Srivastava et al., 2017; Gilbert et al., 2018; Gupta et al., 2022). Perturbations in this homeostatic balance—collectively termed dysbiosis—are strongly correlated with uterine pathologies, diminished semen freezability, repeat breeding, and early embryonic mortality (Jerome and Srivastava, 2012; Pascottini et al., 2020; Yildirim et al., 2026).

Crucially, reproductive microbiota biology is fundamentally a bidirectional dyad between the male and female tracts. Artificial insemination (AI) and natural service do not represent microbially sterile inoculations; rather, ejaculates and extended semen deliver distinct bacterial consortia directly into the female reproductive tract, influencing post-breeding inflammatory cascades and subsequent conception rates (Luecke et al., 2022). Furthermore, advancing our understanding of reproductive microbial dynamics is inextricably linked to the global One Health initiative. Broad-spectrum antimicrobials are often used to treat postpartum uterine infections and male accessory gland pathologies. This directly leads to antimicrobial resistance (AMR) in agricultural settings. Deciphering native commensal communities provides an actionable roadmap toward non-antibiotic interventions, including targeted pre-, pro-, and symbiotic. This review synthesizes current insights into the reproductive microbiota across female and male domestic livestock, evaluates the technical caveats of low-biomass sequencing, provides a comparative multi-species synthesis, and highlights translational pathways for precision animal breeding.

EVOLUTION OF THE STERILE UTERUS PARADIGM: EMERGENCE OF THE REPRODUCTIVE MICROBIOME CONCEPT

Therapeutic and natural deliveries consider the uterus a sterile area. Numerous studies carried out using classical microbiological techniques did not identify any organism where the uterus is involved. This limitation is because these techniques reveal just a small percentage of the variety of microorganisms present, making our understanding of microbial communities associated with a host extremely limited (Gilbert et al., 2018; Baker et al., 2018). At the same time, the discovery of new molecular methods, such as 16S rRNA gene sequencing and shotgun metagenomics, proved that while the traditional beliefs regarding the sterility of uterine tissues might have been true, this approach also provided evidence of the presence of DNA from microorganisms in the tissues previously considered to be sterile (Moreno et al., 2016; Franasia et al., 2016; Baker et al., 2018).

The interpretation of these findings is complicated by the fact that the reproductive tract is a low-biomass space, which means that the microbiome analysis is prone to contamination at the sampling point, DNA extraction, sequencing, and analysis of the samples. Reagent- and extraction-kit-derived contaminants can dominate such samples, and negative controls must therefore accompany every batch and processing step; suboptimal contamination controls, combined with an over-reliance on automated decontamination algorithms, can generate apparently real but spurious taxa (de Goffau et al., 2021; Baker et al., 2019; Davidson et al., 2025). Quantifying absolute microbial biomass is also advisable, because the amount of input biomass strongly influences measurement variance and downstream interpretation (Bender et al., 2018; Salter et al., 2014; Kennedy et al., 2023). It is therefore essential to maintain strict contamination control.

The presence of a stable uterine microbiome is still under dispute, though there is growing evidence that microorganisms play a role in reproductive physiology, immune balance, and susceptibility to diseases, particularly postpartum when the uterus is frequently colonized (Fig. 1) (Sheldon et al., 2019). Modern science is more focused not on whether microorganisms exist, but on whether their interactions with reproductive microbiota, host immunity, endocrine signalling, and environmental factors lead to a more profound understanding of fertility and reproductive health. There have been developments in multi-omics technologies, which are set to increase the understanding of the func-

tions of these microbial ecosystems in the human body and in the diseases (Łaniewski et al., 2022; Zheng et al., 2025).

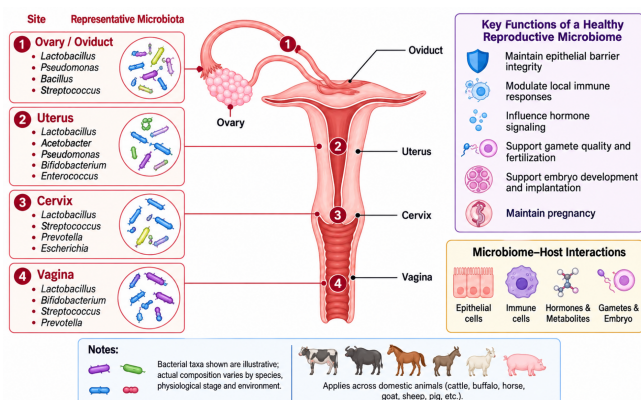


Figure 1: Reproductive tract microbiome and its potential roles in reproductive health across domestic animals. Schematic representation of the representative microbiota associated with different anatomical sites of the female reproductive tract, including the ovary/oviduct, uterus, cervix, and vagina. The composition of the microbiota varies according to reproductive site, animal species, physiological status, and environmental conditions. The reproductive microbiome may interact with epithelial and immune cells, hormones, metabolites, gametes, and embryos to influence epithelial barrier integrity, local immune responses, hormonal signaling, gamete quality and fertilization, embryo development and implantation, and maintenance of pregnancy. The figure provides illustrative bacterial taxa and is intended to represent the general concept across domestic animals, including cattle, buffalo, horse, goat, sheep, and pig. The figure was created using BioRender (BioRender.com) and further refined in Microsoft PowerPoint.

THE FEMALE REPRODUCTIVE TRACT MICROBIOME: AN ECOLOGICAL NETWORK SHAPING FERTILITY

The female reproductive system has been gaining interest lately as a living ecosystem instead of just an organ. The systems involved in reproduction must balance the hormones involved, the immune system, the epithelial system, metabolic products, and the intelligence of the organisms residing in the reproductive system, all of which are important for *in vitro* fertilization, implantation, pregnancy, and postpartum recovery (Gilbert et al., 2018; Baker et al., 2018; Sheldon et al., 2019). The reproductive microbiome of the females differs greatly from that of the gastrointestinal tract, as it keeps changing due to the estrous cycle, pregnancy, age, nutrition, management, and immunity of the host. During the fluctuation of hormones, *i.e.*, the changes in the level of estrogen and progesterone, the chemistries of the vagina, cervix, uterus, and oviduct change as well

(Moreno et al., 2016; Łaniewski et al., 2022). The vagina is the most diverse organ, with many microorganisms being able to live in it due to its anatomical position and constant exposure to the outside environment.

While humans mainly have *Lactobacillus*, pets and livestock contain a greater variety of microorganisms, including genera such as *Corynebacterium*, *Streptococcus*, *Bacteroides*, *Prevotella*, *Fusobacterium*, *Ureaplasma*, and *Clostridium*. Across vertebrates, the reproductive tract microbiota is better described as a continuum of communities along the tract than as a single uniform community, and sites sampled at greater depth (uterus, cervix) yield sparser and more variable communities than the vagina (Chen et al., 2017). The uterine microbiome has become one of the most controversial issues in reproductive biology. While the healthy uterus was regarded as being sterile for a long time, modern studies involving 16S rRNA gene sequencing and shotgun metagenomics show that microbial DNA can be found in reproductive organs and tissues, thus proving the previously accepted theory wrong and shedding light on the difficulties that researchers might face when dealing with low-biomass samples (Baker et al., 2018; de Goffau et al., 2021). There is evidence that reproductive success relies not only on the presence of microorganisms but also on maintaining a properly functioning ecosystem between the host and microbes (Forcina et al., 2022).

When this balance is not restored, the animals may suffer from metritis, clinical and subclinical endometritis, repeated inseminations, and a lower fertility rate (Sheldon et al., 2009; Machado et al., 2012; Jeon et al., 2015; Pascottini et al., 2020). Comparative investigations show significant differences in reproductive microbial communities among cattle, buffalo, mares, pigs, sheep, and goats, taking into consideration their reproductive physiology, hormonal regulation, environmental factors, and management styles (Clemmons et al., 2017). Therefore, modern scientific findings have shifted focus from identifying individual microbial species to understanding the relationships between microbial communities and the host's immune system, hormonal signals, and metabolism to develop innovative concepts for enhancing the fertility of domestic animals.

THE MALE REPRODUCTIVE TRACT AND SEMINAL MICROBIOME: THE NEGLECTED HALF OF REPRODUCTIVE FERTILITY

Composition of the seminal microbiome across livestock species

Although the male reproductive tract was long assumed to contribute little to reproductive microbiology, sequencing-based studies now describe consistent bacterial communities in the semen of farm animals. In bulls, the seminal liquid microbiome is dominated by the phyla Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, and Fusobacteria, and its taxonomic diversity has been linked to fertility and identified as a target for probiotic modulation (Yildirim et al., 2026). In healthy fertile stallions, semen is dominated by *Bacteroidetes* (46.50%), *Firmicutes* (29.92%), and *Actinobacteria* (13.58%), with *Porphyromonadaceae* (33.18%), *Peptoniphilaceae* (14.09%), *Corynebacteriaceae* (11.32%), and *Prevotellaceae* (9.05%) as the most abundant families (Quiñones-Pérez et al., 2021). Comparative sampling of stallions across three European countries confirmed high inter-individual and geographical variability and underlined the practical challenge of defining a “normal” seminal community (Malaluang et al., 2024). In boars, seminal bacterial communities also differ between artificial insemination centres rather than forming a single species-typical profile-*Ruminococcaceae*-dominated communities in some studs versus *Pseudomonadaceae*- and *Enterobacteriaceae*-dominated communities in others (Contreras et al., 2023), and seasonal shifts in composition occur in goat bucks (Mocé et al., 2022).

Seminal microbiota and sperm function

Evidence linking seminal communities to sperm quality is accumulating but remains largely correlative:

- In stallions, the relative abundance of *Peptoniphilaceae* correlates positively with total sperm motility, whereas *Clostridiales Incertae Sedis XI* correlates negatively with progressive motility (Quiñones-Pérez et al., 2022).
- In boars, seminal bacterial composition is associated with sperm quality and fertilizing capacity, with seasonal variation (Zhang et al., 2020).
- In goat bucks, *Sphingomonas* and *Faecalibacterium* have been proposed as candidate biomarkers of semen quality, with composition differing between breeding and non-breeding seasons (Mocé et al., 2022).
- In cattle, seminal microbiome composition has been linked to fertility outcomes and to the quality of stored, extended semen (McAnally et al., 2023; Yildirim et al., 2026).

Origins of seminal microbiota and implications for semen handling

Seminal communities are acquired from the external genitalia, the collection environment, equipment, and personnel, and are further shaped by extender composition and antibiotic supplementation during processing-factors that must be treated as confounders when interpreting semen-microbiome studies (Malaluang et al., 2024; McAnally et al., 2023).

Seminal–female tract interaction after insemination

Artificial insemination is not a microbially neutral event: it deposits semen-borne bacteria into the female tract, where they can interact with the resident vagino-uterine community. Recent cattle studies explicitly modelled the seminal and vagino-uterine microbiomes together and found individual and interactive effects on fertility, indicating that male- and female-side communities should be analysed jointly rather than in isolation (Luecke et al., 2022). This interaction framework is the strongest current argument for treating the seminal microbiome as a reproductive-health component rather than a mere contamination of ejaculates.

Knowledge gaps on the male side

- No consensus core seminal microbiome has been established for any livestock species (Contreras et al., 2023).
- Fertility-linked (as opposed to sperm-parameter-linked) outcomes have scarcely been measured in bulls.
- Mechanisms by which seminal bacteria influence spermatozoa—direct adhesion, metabolite production, or immune activation—remain virtually unexplored (Contreras et al., 2023).

MICROBIAL DIVERSITY ACROSS LIVESTOCK SPECIES

Studies of comparative microbiomes have indicated that livestock species also have differences in the composition and functional characteristics of the reproductive microbiome, which could be attributed to factors such as reproductive anatomy, endocrine physiology, management practices, environmental exposure, and host immune response (Gilbert et al., 2018). Most research on reproductive microbiome in livestock is done on dairy cows, and unprecedented microbial succession is seen in healthy cows throughout the postpartum period following the process of uterine involution (Machado et al., 2012). Any dysbiosis would lead to higher amounts of *Fusobacterium*, *Trueperella*,

Bacteroides, *Porphyromonas*, and *Prevotella* and is associated with diseases like endometritis, metritis, delayed uterine involution, and reduced fertility (Jeon et al., 2015; Sheldon et al., 2019; Pascottini et al., 2020). Yet it should be noted that the advancement of the disease may depend on some other factors, including the immune response of the host, its oxidative stress, metabolic status, and management, which underlines the multifactorial nature of reproductive disorders.

Compared to cattle, the knowledge regarding the reproductive microbiome of buffalo, mare, pig, sheep, and goat is still limited. It is known that, in buffaloes, the changes seen in the microbial composition after giving birth may be similar to those seen in cows; however, continuing research would be needed to link and draw conclusions regarding the composition of microbial communities in buffalo (Kumar et al., 2022). There is emerging evidence that supports the idea that the maternal reproductive microbiome affects the conception, development of the fetus, immunity initiation, and health of the piglet, and the modules that alter the microbial composition include those related to nutrition, probiotics, and housing (Peltoniemi et al., 2021). On the other hand, research into reproductive microbiomes of cattle is only starting, although some studies have already demonstrated that microbial diversity can fluctuate during different stages of pregnancy, labor, and postpartum recovery (Ault-Seay et al., 2023). The significance of this research lies in the fact that the findings stress the importance of the species-specificity of reproductive microbiomes and the necessity of comparing and studying their functioning over time.

FROM ASSOCIATION TO CAUSALITY: METHODOLOGICAL AND CRITICAL APPRAISAL

Confounding factors that must be controlled

Associations between reproductive microbiota and fertility are confounded by parity, stage of the oestrous cycle and pregnancy, metabolic status (including negative energy balance), perineal hygiene at sampling, antibiotic history (systemic and local), season, diet, and housing (Ong et al., 2022; Soffa et al., 2025). Studies that fail to report these covariates cannot support mechanistic claims. Exemplars of stronger design include the case-controlled metritis study that excluded antibiotic-treated cows (Figueiredo et al., 2024) and postpartum work using a standardised cytobrush protocol with perineal cleansing (Pascottini et al., 2020).

Contamination control in low-biomass samples

Because the reproductive tract is low-biomass, reagent and extraction-kit contaminants (“kitome”) can dominate profiles (Salter et al., 2014). Sparse or uneven negative controls combined with automated decontamination algorithms can yield false positives (Kennedy et al., 2023), and absolute biomass should be quantified because input biomass drives measurement variance (Bender et al., 2018). Studies modelling best practices include the use of diluent negative controls in extended semen (McAnally et al., 2023) and the exclusion of taxa that are repeatedly reported as kitome contaminants (e.g., in human endometrial microbiome work) (Moreno et al., 2022). Interpretive limit: 16S rRNA sequencing detects DNA, not viable organisms, so compositional data should not be equated with infection (Baker et al., 2018).

Negative, null, and contradictory findings

Critical appraisal requires reporting where evidence does not support microbiome-mediated fertility loss:

- In dairy cows with metritis, reduced fertility in affected animals was not clearly mediated by key uterine microbiome differences, and failure of clinical cure was unrelated to microbiome shifts (Figueiredo et al., 2024).

- In subclinical endometritis, classical uterine pathogens were essentially absent, with enrichment of *Lactobacillus* and *Acinetobacter* instead—a pattern inconsistent with the simple “pathogen = disease” model (Wang et al., 2018).

- A systematic review of culture-independent studies of the bovine reproductive tract concluded that it remains unclear whether observed dysbiosis is a cause of reproductive disease, a consequence of it, or a sequencing artifact (Ong et al., 2021).

- Longitudinal postpartum studies show delayed differentiation of vaginal and uterine communities in cows that develop endometritis and time-dependent microbial succession in uterine fluid-patterns that cross-sectional designs systematically miss (Miranda-CasoLuengo et al., 2019; Santos & Bicalho, 2012).

Where causal evidence can come from

Causal inference will require culture-based isolation linked prospectively to fertility outcomes—as demonstrated in beef cattle, where cultured uterine bacteria were associated with pregnancy outcomes after AI (Webb et al., 2023), and controlled probiotic interventions, including formulations tested for bovine reproductive health (Gohil et al., 2023) and conceptual frame-

Table 1: . Comparative reproductive microbiome across major livestock species.

Species (site)	Dominant / characteristic taxa	Pathobionts or disease associations	Fertility associations	Main knowledge gap
Dairy cow (uterus, vagina)	<i>Lactobacillus</i> and <i>Acinetobacter</i> enriched in subclinical endometritis (Wang, et al., 2018)	<i>Fusobacterium</i> , <i>Trueperella</i> , <i>Bacteroides</i> , <i>Porphyromonas</i> , <i>Prevotella</i> in metritis/endometritis (Pascottini et al., 2020; Wang et al., 2018)	Impaired conception in diseased cows; fertility loss not clearly microbiome-mediated (Figueiredo et al., 2024)	Causal, intervention-based evidence
Bull (semen)	Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria (Yildirim et al., 2026)	Opportunistic bacteria associated with poorer semen quality (Yildirim et al., 2026)	Seminal–vagino-uterine interaction affects pregnancy outcomes (Luecke et al., 2022)	No consensus core seminal microbiome; few fertility-linked bull studies
Stallion (semen)	Bacteroidetes 46.50%, Firmicutes 29.92%, Actinobacteria 13.58%, Porphyromonadaceae 33.18%, Peptoniphilaceae 14.09%, Corynebacteriaceae 11.32%, Prevotellaceae 9.05% (Quiñones-Pérez et al., 2021, 2022)	High inter-individual and geographical variability (Malaluang et al., 2024)	Peptoniphilaceae ↑ total motility; Clostridiales Incertae Sedis XI ↓ progressive motility (Quiñones-Pérez et al., 2022)	Mechanisms unresolved; no mare data
Boar (semen)	Ruminococcaceae vs Pseudomonadaceae/Enterobacteriaceae depending on stud (Contreras et al., 2023)	Pathogen–commensal interactions in ejaculates (Contreras et al., 2023)	Composition linked to sperm quality; seasonal shifts (Zhang et al., 2020)	Fertility endpoints beyond laboratory sperm parameters
Goat buck (semen)	<i>Sphingomonas</i> , <i>Faecalibacterium</i> (candidate biomarkers) (Mocé et al., 2022)	Seasonal compositional shifts (Mocé et al., 2022)	Semen-quality associations (Mocé et al., 2022)	No ewe/doe reproductive-tract data
Buffalo	Postpartum shifts broadly similar to cattle	Not established	Not established	Longitudinal fertility-linked studies absent
Pig (female tract)	Maternal reproductive microbiome linked to piglet immunity	Not established	Associations with conception and fetal development	Parity/housing effects unquantified
Sheep / ewe	Not established	Not established	Not established	No baseline data

works for vaginal probiotic management of uterine disease and reproductive performance in dairy cows (Adnane et al., 2024).

ENDOCRINE–MICROBIOME–IMMUNE CROSSTALK IN REPRODUCTIVE HOMEOSTASIS

Reproductive functions are activated by the signalling of various hormones, the actions of different microbes, and maternal immune responses. The levels of hormones such as oestrogen and progesterone regulate not only reproductive functions but also the microbial composition and functions of the reproductive tract (Fig. 2). In addition to this, the microorganisms present in the reproductive tract are changed by their products such as short-chain fatty acids and lipopolysaccharides,

which influence the epithelial cells of the reproductive tract (Wira et al., 2015; Baker et al., 2018).

This dynamic makes host–microbe interactions critical for ensuring that the maternal immune system stays tolerant to other aspects of the processes. Beneficial microbes regulate immune functions that influence the activity of regulatory T cells and the function of the inflammatory barrier at the cellular level, thus allowing for proper embryo implantation. Evidence suggests that negative microbiome changes cause overproduction of inflammatory substances such as TNF- α and IL-1 β , which may impede the normal embryo implantation process and prevent the embryo from forming properly. Because inflammation further disrupts microbial homeostasis, a self-perpetuating cycle of dysbiosis and immune activation may contribute to reproductive failure and infertility (Sheldon et al., 2019; Łaniewski et

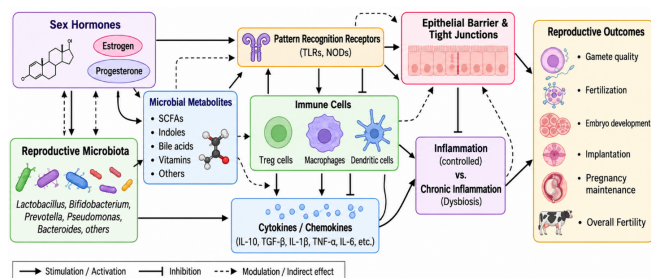


Figure 2: Endocrine-Microbiome-Immune crosstalk in reproductive homeostasis versus disease. Detailed schematic illustrating the bidirectional regulatory networks between ovarian steroid hormones (17 β -estradiol and progesterone), commensal reproductive microbiota, and mucosal immune defense. Commensal taxa process substrates into short-chain fatty acids (SCFAs), indoles, and beneficial metabolites that signal through epithelial pattern recognition receptors (TLRs, NODs) to upregulate tight junction proteins and promote regulatory T-cell (Treg) differentiation and anti-inflammatory cytokine secretion (IL-10, TGF- β). In contrast, dysbiosis triggered by metabolic stress, trauma, or immunosuppression leads to opportunistic pathogen proliferation (*Trueperella*, *Fusobacterium*, *Prevotella*), excess endotoxin (LPS) and pyolysin release, overactivation of TLR4/NF- κ B signaling, chronic pro-inflammatory cytokine expression (TNF- α , IL-1 β , IL-6, CXCL8), severe endometrial epithelial breakdown, embryonic resorption, and systemic sub-fertility. The figure was created using BioRender (BioRender.com) and further refined in Microsoft PowerPoint.

al., 2022).

DYSBIOSIS AND MICROBIOME-BASED STRATEGIES FOR IMPROVING REPRODUCTIVE HEALTH

To maintain reproductive homeostasis, it is vital to support balance in the reproductive microbiome. Disruption of this balance, which is known as dysbiosis, is becoming an increasingly recognized factor in reproductive diseases in livestock. Dysbiosis can be characterized by less diversity among the microorganisms, lack of beneficial microorganisms, and increased numbers of harmful microorganisms. These cause problems in the interactions between host and microorganisms, which leads to inflammation (Gilbert et al., 2018; Łaniewski et al., 2022). Reproductive diseases are not caused by one specific pathogen; diseases occur as a result of interaction between microorganisms, the immune system, endocrine regulation of reproductive function, metabolism, and the environment (Sheldon et al., 2019). The postpartum period is the weakest point of the whole reproductive process since the cervix relaxes, the uterus involutes, tissues reconstitute, and temporary immunosuppression occurs, which al-

lows bacteria to proliferate. While in healthy animals, the restoration of the balance in the microbiome takes place within several hours due to an effective innate immune reaction; the restoration of the balance in the microbiome takes place within several hours due to an effective innate immune reaction, when opportunistic microorganisms, such as *Fusobacterium*, *Trueperella pyogenes*, *Bacteroides* spp., and *Prevotella* spp., are not eliminated, animals are at risk of being affected by diseases such as metritis, endometritis, and ineffective breeding cycles (Machado et al., 2012; Jeon et al., 2015; Pascottini et al., 2020). Increasing evidence also suggests that subclinical dysbiosis may contribute to repeat breeding syndrome by altering sperm transport, embryo implantation, maternal immune tolerance, and endometrial receptivity in the absence of overt clinical disease.

An increasing comprehension of microbial reproductive ecology is quickly changing veterinary reproductive medicine from simple microbiology to accurate diagnosis and effective treatment. Innovations in next-generation sequencing, quantitative PCR, metagenomics, metabolomics, transcriptomics, and machine learning have made it possible to identify microorganisms related to uterine health, conception, embryo viability, and postpartum diseases, providing a chance for timely diagnosis and custom reproductive treatment (Baker et al., 2018; de Goffau et al., 2021). At the same time, the reproductive microbiome becomes an important factor for ARTs, such as artificial insemination, embryo transfer, ovum pick-up, IVF, and embryo cryopreservation, with microbial composition having an impact on semen quality (Srivastava et al., 2013; Singh et al., 2015), embryo viability, implantation, and pregnancy outcome (Moreno et al., 2016; Łaniewski et al., 2022). These advancements have raised interest in microbiome modulation with the help of pre-, pro-, and symbiotic and targeted antimicrobial therapy that seeks to treat disorders by restoring microbial balance without the use of antibiotics. Interventions aimed at restoring microbial balance remain largely experimental (Bicalho et al., 2017; Sheldon et al., 2019). Probiotic formulations have been assessed for their ability to augment reproductive health in cattle, with in vitro, in silico, and in vivo evidence of modulation (Gohil et al., 2023), and vaginal probiotic strategies for the management of uterine disease and reproductive performance in dairy cows have been conceptually reviewed (Adnane et al., 2024). In the male, seminal microbiota has been proposed as a target for probiotic modulation to improve fertility parameters (Yildirim et al., 2026). None of these approaches yet rests on demonstrated

causal mechanisms, and they should be presented as a research agenda rather than an established therapy.”

FUTURE PERSPECTIVES: ARTIFICIAL INTELLIGENCE AND PRECISION REPRODUCTIVE MICROBIOME RESEARCH

The merging of AI, multi-omics technologies, and research on the reproductive microbiome is transforming veterinary reproductive practices. The massive data set produced by high-throughput sequencing contains information on many microbial species and their functional genes, metabolites, hormones, records of reproduction, and immune responses of the host, forming a collection that cannot be analysed using traditional statistical methods. Machine learning and AI algorithms can allow integration of this multi-dimensional data set to discover microbial tissue markers responsible for fertility, postpartum uterine diseases, embryo survivability, semen quality, and pregnancies, allowing for predictive diagnostics and innovative pregnancy management (Gilbert et al., 2018; Baker et al., 2018; Topol, 2019). In animal farming, decision-support platforms using AI could provide a way to discover animals suffering from reproductive diseases earlier, help with the selection of animals for embryo transfer, predict the success rate of artificial insemination, and utilize microbial fingerprints in assessing bulls for fertility, contributing additional data to the traditional practice while ensuring scientifically based decisions (Łaniewski et al., 2022). “Equally, AI-based integration of seminal microbiome data with conventional sperm-quality assessment could support bull fertility evaluation, though the absence of a defined core seminal microbiome currently limits such models (Contreras et al., 2023; Yildirim et al., 2026)”.

However, despite the rapidly increasing body of research driven by the compelling socioeconomic reasons mentioned above, several scientific and methodological obstacles still need to be overcome for the successful implementation of reproductive management based on microbiome studies. Reproductive tissues have a small biomass, which poses the risk of sample contamination during sampling, DNA extraction, and sequencing; therefore, there is a need to ensure strict quality control and established bioinformatic work procedures to enable microbiome studies (Baker et al., 2018; de Goffau et al., 2021). Moreover, many recent studies have explored the links between microbial dysbiosis and reproductive disorders rather than the causal relationships between them. This situation suggests the need for further longitudinal studies, intervention

studies, and experiments to better understand the host-microbe interactions taking place. There is substantial variability between species in their reproductive morphology, endocrine physiology, immunological control, management, and environmental exposure, which raises doubts about the applicability of findings derived from studies on humans or animals raised in laboratories to livestock (Sheldon et al., 2019). To overcome these challenges, it is essential to combine the efforts of microbiologists, reproductive biologists, immunologists, genomicists, bioinformaticians, and AI scientists so that discoveries in microbiology can serve as a foundation for developing reliable diagnostic methods, targeted therapies, and precise reproductive strategies aimed at enhancing fertility, conserving antibiotics, and implementing the One Health approach.

CONCLUSION

Recently, the reproductive microbiome has emerged as one of the fastest-growing and most exciting fields in reproductive biology. Once considered only as a source of infection, microbial populations are now considered as active contributors to the regulation of reproductive processes. Even though many questions remain unanswered, available data strongly suggest that reproductive success is determined not only by hormones and genetics but also by the state of the microbiome in the reproductive tract. Dysbiosis of the reproductive tract is associated with uterine diseases, reduced semen quality, repeat breeding, failure to implant, and the loss of early embryos in different domestic animals. The seminal microbiome is now recognised as a partner of the female tract microbiome in determining the outcome of insemination, although the two are rarely studied together (Luecke et al., 2022).

Overall, the vast body of literature examined in the present review allows us to conclude that the field is moving from purely descriptive microbiology to a more practical application of microbiological knowledge. The latest developments in sequencing, multi-omics, bioinformatics, and AI allow scientists to create the basis for applications of microbiome diagnostics and targeted therapy. However, microbiome data should be treated with caution since the differences in methodologies, low content of microorganisms in the samples, and species variations remain significant problems. For veterinarians, livestock producers, and reproductive biologists, the reproductive microbiome is more than just an academic curiosity. This new biological approach could help in understanding, assessing and potentially increasing the reproductive capacity.

This area of research will gain importance in reproductive biotechnology and precision veterinary medicine and will lead to healthier animals while increasing the potential for reproduction and making livestock production more sustainable.

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