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Reproductive Microbiota and Swine Fertility: A New Perspective on Semen Quality

Microbiota reprodutiva e fertilidade em suínos: uma nova perspectiva sobre a qualidade do sêmen

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Abstract

Recent studies on the characterization of the seminal microbiota have identified key factors associated with animal fertility, particularly influencing sperm quality. The use of Next Generation Sequencing (NGS) technologies and the development of advanced analytical tools have allowed for a more comprehensive characterization of the microbial communities present in semen, revealing a much greater diversity than previously anticipated. These methodologies have made it possible to investigate microorganisms that are either present in low abundance or difficult to culture, as well as to explore the composition and abundance of these populations under different conditions. Within this context, the objective of this work was to describe and discuss the main advances regarding: (i) microbial characteristics associated with sperm quality; (ii) the effects of the gut microbiota on male reproductive performance; (iii) external factors involved with the modulation of these communities; and (iv) practical implications and future perspectives for swine reproduction.

Keywords: *Microbiota, Seminal plasma, Swine Fertility*

Introduction

The seminal microbiota has been recognized as one of the major factors influencing sperm quality and reproductive efficiency in boars. Advances in omics technologies particularly with the development of Next Generation Sequencing (NGS) platforms and bioinformatics tools for data analysis have enabled a more comprehensive and accurate characterization of the seminal microbiota, including the definition of taxonomic profiles detailing the bacterial communities present in this environment. This approach has not only allowed the identification of a greater diversity of microorganisms, including those that are difficult to culture or present in low abundance, but also facilitated the investigation of important ecological aspects, such as species richness and variations between different samples or experimental groups. Consequently, it has become possible to better understand the microbial dynamics within the seminal environment and their potential implications for reproductive health and animal performance.

Recent studies have demonstrated that the composition and diversity of microbial communities in the male reproductive tract directly influence sperm parameters (Gòdia et al., 2020; Zhang et al., 2020). Beyond the local microbiota, emerging evidence has highlighted the importance of the gut–testis axis, wherein bacterial metabolites and inflammatory signals originating from the gut microbiota may impact sperm function (Chen et al., 2024). Additionally, extrinsic factors such as nutritional management, environmental conditions, and the use of antimicrobials can modulate these microbial communities, creating new opportunities for strategic interventions aimed at improving reproductive outcomes (Han et al., 2022; Ngo et al., 2023).

Given the relevance of the microbiota into the reproductive efficiency in swine production, this article aims to synthesize the current knowledge on: (i) microbial characteristics associated with sperm quality; (ii) the effects of the gut microbiota on male reproductive performance; (iii) external factors involved with the modulation of these communities; and (iv) practical implications and future perspectives for swine reproduction.

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Seminal microbiota profile and its association with sperm quality biomarkers

Although increasing evidence suggests that interactions between the microbiota and spermatozoa play a significant role in boar fertility, as well as in the transmission of pathogens and antimicrobial resistance genes, the characterization of the swine seminal microbiome remains limited. This limitation is primarily due to the numerous variables that can influence microbiome assessments (Gòdia et al., 2020).

The seminal microbiota of boars exhibits a complex and dynamically regulated composition, with important implications for reproductive performance. Microbial sequencing analyses have consistently identified the predominant bacterial phyla present in the swine seminal microbiota, revealing a dominance of Proteobacteria (9.80%–60.00%), Firmicutes (22.50%–60.91%), Actinobacteria (10.10%–27.93%), and Bacteroidetes (4.24%–30.43%), as summarized in Table 1. These variations, observed both across different studies and among samples within the same experiment, underscore the highly dynamic nature of the seminal microbial community, which responds to environmental, physiological, and management-related factors. Notably, these microbial populations coexist with spermatozoa, engaging in complex interactions that can directly influence sperm functionality and, consequently, reproductive outcomes.

Several studies have emphasized the relevance of the *Proteobacteria* phylum in the composition of swine seminal microbiota and its possible association with sperm quality. Within this phylum, several bacteria are known to be detrimental to swine sperm, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Serratia marcescens*, and *Proteus* spp. Genera such as *Pseudomonas*, *Escherichia/Shigella*, *Acinetobacter*, and *Burkholderia* have been identified as predominant in different contexts (Gòdia et al., 2020; Zhang et al., 2020; Ngo et al., 2023; McAnally et al., 2023). The presence of *Pseudomonas aeruginosa* in swine semen has been linked to detrimental effects on sperm quality. Notably, a high abundance of *P. aeruginosa* in samples collected during the summer was correlated with decreased sperm motility and increased rates of morphological abnormalities. Moreover, functional prediction analyses of the microbial community revealed that seminal microbiota dominated by *P. aeruginosa* was associated with metabolic pathways related to oxidative stress, suggesting a potential mechanism through which this bacterium compromises sperm integrity (Zhang et al., 2020).

In the study by Gòdia et al. (2020), using RNA-Seq technology revealed a diverse bacterial composition, including representatives of the genus *Acinetobacter* in the swine seminal plasma. Although this genus was identified as a differential marker between ejaculates of varying seminal quality, the authors did not establish a direct association between *Acinetobacter* and reduced sperm viability or alterations in sperm membrane integrity.

Ngo et al. (2023) observed a positive association between the presence of *Delftia acidovorans*, a member of the *Proteobacteria* phylum, and swine semen quality. This species was correlated with improvements in sperm motility and viability. The authors noted that although *D. acidovorans* represents only a small percentage of the seminal microbiota, suggesting its potential as a positive biomarker for boar fertility. Although *Proteobacteria* are part of the normal seminal microbiota, the excessive abundance of key microorganisms within this phylum may reflect microbial imbalance, indicating dysbiosis and a potential impact on fertility. Thus, the characterization and monitoring of bacterial composition, especially members of this phylum, may serve as an essential tool for assessing reproductive health in boars.

The *Firmicutes* phylum is also consistently present in significant proportions across all the studies on swine microbiota composition. According to Gòdia et al. (2020), this phylum was the most abundant identified in swine semen, highlighting its active role in the seminal fluid. The presence of bacteria belonging to the *Ruminococcus* genus, from the *Firmicutes* phylum, for example, was negatively correlated with sperm motility. Zhang et al. (2020) also identified the predominance of *Firmicutes* among bacterial communities found in swine semen, observing that different compositions within this phylum was correlated with various sperm parameters, such as motility and DNA integrity. On the other hand, bacteria such as *Lactobacillus* spp., also part of the *Firmicutes* phylum, are often recognized for their probiotic potential, and were suggested to maintain sperm quality. Ngo et al. (2023) corroborate the findings of Zhang et al. (2020), reporting that a higher relative abundance of *Firmicutes* was associated with better sperm motility and viability scores. The study suggests that changes in the composition of this phylum may reflect adaptations to the environment and management practices, directly influencing animal fertility. Furthermore, McAnally et al. (2023b) identified the presence of *Firmicutes* in the reproductive tract of prepubertal boars, suggesting that this phylum is present from early stages of reproductive development and may participate in the maturation of the spermatic environment. Therefore, the *Firmicutes* phylum, composed of various bacterial genera, appears to be closely associated with sperm functionality in swine, potentially acting both beneficial and detrimental, depending on its specific composition.

Table 1. Comparative characterization of the swine seminal microbiota: Phyla, Genera, and main findings

Reference	Location	Dominant Phyla	Relevant Genera or Species	Main Findings
Gòdia et al. (2020)	Spain	Proteobacteria (39.1%), Firmicutes (27.5%), Actinobacteria (14.9%)	*Bacillus megaterium* (4.3%), *Brachybacterium faecium* (3.3%), *Bacillus coagulans* (1.2%)	RNA-Seq analysis. Most abundant bacteria were environmental, suggesting post-ejaculation contamination.
Zhang et al. (2020)	China	Proteobacteria (57.53%), Firmicutes (31.17%), Bacteroidetes (4.24%)	*Pseudomonas* (34.41%), *Lactobacillus* (19.93%), *Ralstonia* (6.8%)	Higher microbial diversity is observed in winter. *Lactobacillus* positively correlated with sperm quality and reproductive performance.
McAnally et al. (2023)	USA	Proteobacteria (47.2–60%), Firmicutes (22.5–31.9%), Actinobacteria (10.1–11.5%)	*Mannheimia* (6.87%), *Psychrobacter* (6.40%), *Moraxella* (6.11%)	Correlations between motility and relative abundance of *Prevotella* ($r = -0.29$), *Ruminococcus* ($r = -0.24$), and *Bacteroides* ($r = -0.32$).
Ngo et al. (2023)	Thailand	Firmicutes (49.5%), Proteobacteria (22.6%), Actinobacteria (12.5%)	*Lactobacillus* (11.8%), *Escherichia–Shigella* (6.2%)	Key microorganisms and variations in minor microbial populations (e.g., *Globicatella sanguinis*, *Delftia acidovorans*, *Alysiella*, *Myroides*) have more impact on semen quality than the overall bacterial profile.
Scherer et al. (unpublished data)	Brazil (RS)	**1st Collection:** Firmicutes (60.91%), Actinobacteria (27.93%), Proteobacteria (9.80%) **2nd Collection:** Firmicutes (45.03%), Bacteroidetes (30.43%), Proteobacteria (13.97%)	**1st Collection:** *Streptococcus* (19%), *Cutibacterium* (18%), *Unclassified Neisseriaceae* (9%) **2nd Collection:** *Bacteroides* (20%), *Clostridium* (17%), *Unclassified Neisseriaceae* (14%)	Changes in the seminal microbiota of the same males over short intervals during summer. Significant shift in *Bacteroidetes* abundance ($FDR < 5 \times 10^{-7}$).

Despite its presence being reported in several studies, little is known about the effects of bacteria from the *Actinobacteria* phylum. McAnally et al. (2023) reported that the relative abundance of *Actinobacteria* varied between two swine reproduction centers, being higher in center A (11.5%) compared to center B (10.1%), with a significant difference ($P = 0.05$). Zhang et al. (2020) observed seasonal variations in the bacterial composition of semen, with differences in the relative abundance of *Actinobacteria* between samples collected in summer and winter; however, none of the studies conducted more in-depth analyses regarding this phylum.

McAnally et al. (2023) identified negative correlations between the relative abundance of *Bacteroidetes* and sperm motility. Specifically, the genera *Prevotella* and *Bacteroides*, belonging to this phylum, showed negative correlations with sperm motility ($r = -0.29$ and $r = -0.32$, respectively). These findings suggest that a higher presence of *Bacteroidetes* in swine semen may be associated with reduced sperm quality, particularly in terms of motility.

Collectively, these findings reinforce the notion that the seminal microbiota constitutes a complex and sensitive ecosystem, with the potential to serve as an indicator of reproductive health and a biomarker of fertility in boars. Ongoing and comprehensive characterization of these microbial communities may support the development of more precise and individualized management strategies, ultimately contributing to the selection of breeding males with superior reproductive performance and potential.

The Role of Gut Microbiota in Regulating Male Reproductive Function

The gut microbiome plays a pivotal role in animal fertility through a complex network of interconnected mechanisms that encompass hormonal regulation, immune balance, and the preservation of reproductive organ integrity. A growing body of research has introduced the concept of the gut–testis axis, a functional link between intestinal microbiota and testicular activity as a key element in this process. This axis helps explain how imbalances in microbial communities may negatively affect spermatogenesis, hormone synthesis, and semen quality. Clinical studies in both humans and murine models have shown that shifts in gut microbiota composition can influence the secretion of sex hormones such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone, thereby modulating the hypothalamic–pituitary–testicular axis. This regulation involves signaling molecules like leptin and ghrelin, whose levels are known to be shaped by gut microbial dynamics (Wang and Xie, 2021). Therefore understanding this intricate relationship offers new perspectives for improving reproductive performance through microbiota-centered approaches.

In addition to its role in endocrine regulation, gut microbiota plays a central role in immune system modulation, contributing to the maintenance of inflammatory homeostasis within the gut–testis axis. According to Wang and Xie (2022), gut microbiota dysbiosis alters the function and anatomy of the intestinal barrier, resulting in low-grade inflammation, metabolic disorders, endocrine dysregulation, and insulin resistance, all of which affect spermatogenesis. Immune dysregulation associated with dysbiosis promotes the release of endotoxins and the activation of inflammatory pathways that directly impact testicular and epididymal tissues. This process can lead to the disruption of the blood–testis barrier (BTB), which is essential for protecting germ cells against immune and environmental threats, and contribute to the development of inflammatory conditions such as orchitis (Wang and Xie, 2022). BTB integrity is partially maintained by gut microbiota-derived metabolites, such as short-chain fatty acids (SCFAs), which also regulate cellular pathways in the testicular environment, directly influencing spermatogenesis and testicular morphology (Lv et al., 2024).

The female reproductive function is coordinated by the endocrine system driven by the hypothalamic-pituitary-gonadal (HPG) axis (Zanella et al., 2024). The endometrial microbiome is composed of a variety of microorganisms that appear to interact dynamically with the hormonal and immune regulation of the female reproductive tract (Zanella et al., 2024). In sows, the gut microbiota contribute to the production of SCFAs and other metabolites crucial for maintaining reproductive health. For example, higher concentrations of SCFAs have been associated with improved reproductive performance in sows, indicating a link between microbial metabolic activity and fertility (Miura et al., 2024).

Jiang et al. (2023) have identified a significant correlation between testosterone levels and gut microbiota composition alterations in Meishan boars. In vitro assays demonstrated that testosterone can modulate microbial structure, whereas specific bacteria are capable of degrading this hormone in a dose-dependent manner. However, whether this bidirectional interaction is a determining factor in hormonal fluctuations and microbiome development remains unclear.

Guo et al. (2020) observed taxonomic and functional alterations in the gut microbiota of Duroc boars with high (group H, 100%) and low (group L, <80%) semen utilization rates. The genera Ruminococcus and Sphingobium were found in higher proportions in group L animals and were negatively correlated with semen utilization. Conversely, RFN20 and Paludibacter were more abundant in group H, with RFN20 showing a statistically significant positive correlation with semen utilization rate. Moreover, functional metabolic differences in gut microbiota were observed between the groups, particularly in the production of branched-chain fatty acids (BCFAs). Individuals with low semen utilization showed significantly elevated plasma levels of endotoxin, zonulin, diamine oxidase, and lipocalin-2, along with a trend toward increased inflammatory cytokines IL-6 and TNF-α.

Additional studies have demonstrated variations in the gut microbial composition and its association with semen quality and reproductive performance in boars. Chao et al. (2024) found that increased presence of bacterial genera such as *Stenotrophomonas* and *Clostridium sensu stricto 3* was related to improved sperm quality, highlighting the functional role of microbiota in fertility modulation.

In the field of therapeutic strategies, probiotic candidates have emerged as a promising alternative for managing seminal microbiota. The use of *Lactobacillus* spp. may be beneficial, particularly due to its positive effects on sperm motility and integrity (Ngo et al., 2023). In mice, its use demonstrated improved semen parameters and testosterone levels, as well as reduced systemic inflammation and enhanced intestinal mucosal integrity (Poutahidis et al., 2014). Additionally, alginate oligosaccharides, a prebiotic, have shown efficacy in promoting favorable gut and seminal microbiota changes, positively impacting semen quality (Han et al., 2022).

Although these associations reinforce the relevance of gut microbiota in male swine reproductive health, the exact mechanisms underlying these interactions remain incompletely understood. The complexity of the pathways involved, including the interplay between microbial metabolites and host physiology, requires further investigation. Elucidating and modulating the Testis–Gut Microbiota Axis may represent a promising strategy to improve male fertility and semen quality (Chen et al., 2024). As in other species, therapeutic interventions targeting gut microbiota modulation, such as probiotics and prebiotics, present promising potential for enhancing male fertility in pigs. This approach stands as an innovative research frontier with significant implications for animal reproduction and the advancement of swine biotechnology.

Environmental and Management Factors Influencing Seminal Microbiota

The environment, management practices, and health status of pig farms significantly impact the diversity of swine microbiota and, consequently, seminal microbiota (Marín et al., 2024). Those factors play a crucial role in shaping microbial communities. For example, higher bacterial diversity has been observed during winter compared to summer, with *Lactobacillus* being more abundant in winter, positively correlating with improved sperm quality and reproductive performance (Zhang et al., 2020). The same research group also demonstrated that the genus *Pseudomonas* is associated with decreased semen quality and fertility, especially during the summer months, indicating a possible seasonal influence on microbiota composition and its impact on fertility (Zhang et al., 2020).

Besides the climate, the geographical regions also can influence the microbiota composition. In tropical conditions, there is a predominance of the phylum *Firmicutes* (49.5%) (Ngo et al., 2023), while in temperate climate, a higher prevalence of *Proteobacteria* (39.1%) is observed, as described by Gòdia et al. (2020). In unpublished data from our research group, semen samples collected from boars housed in a climate-controlled artificial insemination center located in the state of Rio Grande do Sul, Brazil (classified as a humid subtropical climate), during the months of December (early summer) and February (peak summer), also showed *Firmicutes* as the predominant phylum (60.91% and 45.03%, respectively). Other phyla showed variations between collections: in Collection 1, *Actinobacteria* (27.93%) and *Proteobacteria* (9.80%) were most abundant; in Collection 2, *Bacteroidetes* (30.43%) and *Proteobacteria* (13.97%) predominated. Statistical analysis revealed a significant difference in *Bacteroidetes* abundance between collections ($FDR < 5 \times 10^{-7}$), suggesting a possible variation in ejaculate microbiota over time or in response to distinct environmental or physiological conditions between collection periods.

At the genus level, Collection 1 (early summer) was characterized by higher abundances of *Streptococcus* (19%), *Cutibacterium* (18%), and unclassified representatives of the *Neisseriaceae* family (9%). In Collection 2 (peak summer), *Bacteroides* (20%), *Clostridium* (17%), and again unclassified members of *Neisseriaceae* (14%) were predominant. Differential abundance analysis between collections identified six genera with significant differences ($FDR < 0.02$): *Cloacibacterium*, *Bacteroides*, *Terrisporobacter*, *Rhodococcus*, *Corynebacterium*, and *Turicibacter*. The identification of these differentially abundant genera reinforces the hypothesis that the boar ejaculate microbiota is dynamic and may be linked to important aspects of reproductive health, semen quality, and potentially, fertility. These findings highlight the importance of longitudinal and comparative studies to better understand the microbial ecology of the swine reproductive tract and its possible zootechnical implications. Combined with data from Ngo et al. (2023) regarding microbial adaptations to different climates, these results underscore the need for region-specific protocols for the control of seminal microbiota.

In addition to seasonal variation, there is also a clear differentiation related to the animals' age. Li et al. (2023) identified notable differences in the diversity and composition of seminal microbiota between boars of different ages (10 vs. 36 months), with older animals showing lower microbial diversity and higher abundances of *Aerococcus*, *Gallicola*, *Ulvibacter*, and *Proteiniphilum* compared to younger pigs. These changes in seminal microbiota were associated with a reduction in the semen's antioxidant capacity, ultimately leading to a decline in quality. In younger animals, the three most abundant bacterial genera were *Streptococcus* (0.159%), unclassified members of the *Corynebacteriaceae* family (0.075%), and unclassified representatives of the *Clostridiales* order (0.097%). These genera mostly belong to the phylum *Firmicutes*, except for *Corynebacteriaceae*, which is part of *Actinobacteria*. In contrast, in older animals, the most prevalent genera were *Atopostipes* (0.150%), unclassified *Clostridiales* (0.130%), and *Porphyromonas* (0.054%). Two of these genera belong to *Firmicutes*, while *Porphyromonas* is affiliated with *Bacteroidetes* (Li et al., 2023).

As it is not feasible to collect boar semen in a completely sterile manner on a large scale, management and hygiene practices directly influence the bacterial community that becomes associated with the ejaculate during collection and processing. Higher levels of contamination are found in semen doses produced under inadequate hygienic conditions, leading to reduced sperm motility and viability (Goldberg et al., 2017). A study by Rocha et al. (2024), which audited 11 boar studs, found that failures in collection and equipment sanitation were associated with deteriorated water and semen quality and increased contamination risks. The authors recommend continuous monitoring, proper hygiene, and staff training as essential measures to ensure semen dose quality. Another major factor is the use of antimicrobials. For years, antimicrobials were employed in swine farming as growth promoters. Considering the relevance of gut and seminal microbiota to sperm quality in boars, antimicrobial use significantly impacts these microbial ecosystems. While effective in preventing disease and promoting animal growth, antimicrobial administration has been associated with increased abundance and diversity of antimicrobial resistance genes (ARGs) in the swine microbiome. Moreover, antimicrobial use in pigs has been shown to alter intestinal microbial diversity. According to Pillay & Abeel (2025), antimicrobial administration accounts for approximately 20% of the variation in bacterial species diversity between pigs raised with and without exposure to these drugs. These alterations not only compromise the microbial balance in the gut and potentially the seminal microbiota but also increase the risk of ARG dissemination in the environment and to other hosts.

Due to the wide variability of boar semen microbiota and the limited understanding of its behavior across climates, environments, and nutrition, it has become standard practice to include antimicrobials in commercial semen extenders to preserve sperm quality. Since boar semen yields better storage results when refrigerated at 15–18°C, a temperature range in which bacterial growth is not completely inhibited, antimicrobials are used to control bacterial proliferation during storage. Although effective, this practice poses significant risks related to antimicrobial resistance and has a major impact on microbiota composition.

Exploring alternatives such as antimicrobial peptides, probiotics, and semen cooling protocols at 4°C offers promising avenues to maintain semen quality and fertility outcomes while mitigating these risks. However, implementing such alternatives requires careful evaluation of their effects on sperm viability and microbial balance.

While reducing antimicrobial use in boar reproduction is essential for combating resistance, it is equally important to consider the broader context of animal health and welfare. Transitioning to low-antimicrobial systems must be carefully managed to prevent compromising animal well-being. Furthermore, the development of resistance is shaped by a complex interplay of genetic, environmental, and management factors, underscoring the necessity for a multifaceted approach to its control. Therefore, understanding the seminal microbiota, its intrinsic characteristics, how environmental and management factors influence it, and how it interacts with both the male reproductive tract and the female uterine microbiota post-insemination is a vital area of current and future research. Investigating the role of seminal microbiota, developing alternatives to antimicrobials in semen extenders, mitigating antimicrobial resistance, and improving overall reproductive health in swine are essential steps toward fostering a more sustainable production system.

Final Considerations and Future Perspectives

These collective findings underscore the crucial role of seminal microbiota in regulating sperm function in boars, especially for the development of targeted microbiological management strategies. A deeper understanding of these microbial interactions paves the way for significant advancements in the reproductive efficiency of breeding herds. However, methodological challenges and the need for local adaptations highlight the importance of continued research.

The central role of seminal microbiota in swine reproductive health also presents new opportunities for interventions. The reduction in antimicrobial use, probiotic supplementation, nutritional adjustments, and enhanced hygiene practices all represent promising avenues for optimizing semen quality, reducing instances of suboptimal fertility, and strengthening artificial insemination programs. Future research should prioritize the practical, cost-effective application of these approaches, with a focus on their successful large-scale implementation in swine production systems.

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