


COMMENTARY

Special series on the role of the microbiome in reproduction and fertility

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This paper forms part of a special series on the Microbiome. The guest editors for this series are Dr Siobhain M O'Mahony (University College Cork, Ireland) and Dr Pierre Comizzoli (Smithsonian's National Zoo and Conservation Biology Institute, USA).

Abstract

Many parts of the animal and human body host groups of bacteria, viruses, and fungi that together are known as the microbiome. Microbiomes do not cause disease but are important for the healthy working of many systems in the body, including for reproduction and fertility. While the microbiome that lives in a reproductive tract play the most direct role, microbiomes from other areas of the body may also affect reproductive health. However, not much is known about how these groups of microorganisms regulate fertility as well as the health of parents and offspring and help animals to cope with environmental changes. Furthermore, compared to the large amount of research in laboratory species and humans, there is less information about domestic or wild animal species. This special series of *Reproduction and Fertility* on microbiomes is aimed at filling this gap with articles from experts highlighting important evidence in reproductive microbiomes, current research gaps, and new directions.

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Introduction

Microbiomes are diverse communities of microorganisms (including bacteria, fungi, viruses, and phages) residing in various niches (e.g. gut, oral cavity, skin, and vagina) in and on living organisms. They play crucial roles in many aspects of health and influence disease as well as disease risks. In recent decades, great research strides have been made to characterize the influence of different communities of microbiota on reproduction and fertility in both human and animal species (Hummel *et al.* 2022). Different microbiomes in the reproductive tract interact with and regulate physiological functions that are related to reproduction and fertility even before conception. This even includes attraction between individuals through secondary sexual features (Rowe *et al.* 2020). Moreover,

the quality of gametes and the reproductive tract environment is very much dependent on a healthy microbiome during and shortly after conception. It then encompasses the optimal development of the fetus and microbiome seeding at birth (Sun *et al.* 2023). In many animal species, the perinatal stage is known to be a key period for microbiome influences on both maternal health and appropriate colonization of the infant gut. In mammals, the milk microbiome also plays a key role during the lactation (Keady *et al.* 2023) to influence the microorganisms that take up residence in the newborn gut.

This special series of the journal *Reproduction and Fertility* explores the intricate relationship between different microbiome niches and reproductive health,

shedding light on mechanisms and implications of their interaction. Although many areas remain to be addressed, the special series provides new insights about microbiomes in reproductive tracts, milk microbiomes, the link between microbiota and reproductive pathologies, and the possibility to manipulate or engineer microbiomes to treat diseases and medical conditions. It also explores comparative aspects and plasticity of microbiomes in wild and domestic animal species.

'Reproductive' microbiomes

The microbiome has been shown to impact gamete development, including spermatogenesis and oogenesis. Specifically, studies in mice have demonstrated that the gut microbiome is capable of influencing the expression of genes related to spermatogenesis and steroidogenesis (Ding *et al.* 2020, Diviccaro *et al.* 2022). This interplay may affect hormone levels and reproductive outcomes. Similarly, suboptimal vaginal microbiome has been associated with impaired oocyte quality and reduced fertility in women (Xu *et al.* 2020).

The microbiome of the reproductive tracts in both males and females contribute to maintaining homeostasis and protecting against pathogens. In females, the vaginal microbiome helps regulate pH and prevent the overgrowth of potentially harmful microorganisms, which can affect fertility and increase the risk of adverse pregnancy outcomes (Moumne *et al.* 2021). In males, the prostate and seminal microbiomes are thought to influence sperm function and reproductive success (Farahani *et al.* 2021).

The microbiome is also noted to have implications for assisted reproductive technologies such as *in vitro* fertilization (IVF). Studies have shown that the bacterial composition of the vaginal microbiome can influence the success rate of IVF procedures, with specific microbial profiles associated with improved implantation and pregnancy rates (Frasasiak *et al.* 2021). Furthermore, emerging evidence suggests that the microbiome of the embryo culture medium used during IVF may impact embryo development and viability (Cariati *et al.* 2023).

During pregnancy, the maternal microbiome undergoes dynamic changes to allow greater energy harvesting and decreased immune response that influence maternal health and fetal development. Imbalances in the gut microbiome have been linked

to gestational complications, such as preeclampsia and gestational diabetes (Stupak & Kwaśniewski 2023). Furthermore, specific bacterial species have been linked to preterm birth independently of clinically apparent infection, uterine anomalies, and other pregnancy complications (Vidmar Šimic *et al.* 2023). Additionally, the placental microbiome has been a subject of investigation but also controversy, with some studies identifying potential implications for fetal immune development and long-term health (Stupak & Kwaśniewski 2023) while others have highlighted the inconsistencies in this research (Walter & Hornef 2021).

The microbiome can also have transgenerational effects on reproductive health. Evidence from animal studies suggests that perturbations in the maternal microbiome can impact the microbiome of offspring, influencing their reproductive outcomes and susceptibility to diseases later in life (Russell *et al.* 2023). Further research is still needed to elucidate the mechanisms underlying these transgenerational effects.

Commonalities and differences between animal species (including humans)

Various animal species harbor diverse bacterial communities in their reproductive tracts, including the vagina, uterus, and seminal fluid. These communities contribute to maintaining a balanced microbial ecosystem critical for reproductive health. For example, females from many animal species, including humans, display a dominance of *Lactobacillus* spp. in their reproductive tract (Chen *et al.* 2021). Lactobacilli have several beneficial properties including contributing to an acidic environment, preventing the overgrowth of pathogenic microorganisms, and promoting sperm survival (Souza *et al.* 2023). Microbes in the reproductive tract can influence fertility by modulating hormone levels, immune responses, and nutrient availability (Moreno & Simon 2019). For instance, certain bacteria produce metabolites that regulate ovulation (Liu *et al.* 2023) or affect sperm motility (Farahani *et al.* 2021).

Anatomical differences between animal species lead to variations in the structures of reproductive tracts. This, in turn, influences the composition and diversity of their reproductive microbiome. The presence of a cloaca in nonmammalian versus mammalian species is a good example (Videvall *et al.* 2018). Moreover, diet is one of the most impactful influences

of the microbiome (Dahl *et al.* 2020), and dietary preferences of animal species impact their microbiome composition (Pilla & Suchodolski 2021), including those involved in reproduction. Herbivorous species may have distinct microbial profiles compared to carnivorous or omnivorous species, affecting reproductive functions (Ley *et al.* 2008, Wu *et al.* 2022). Interestingly, animal species with different modes of reproduction, such as internal fertilization, external fertilization, or asexual reproduction, exhibit variations in their reproductive microbiome. For example, species engaging in internal fertilization often have more complex microbial communities in their reproductive tracts (Rowe *et al.* 2020). Lastly, each animal species has evolved unique interactions with its microbiome, leading to species-specific microbial compositions. These interactions are influenced by factors such as genetics, behavior, and environment, resulting in distinct microbial signatures in the various reproductive microbiomes (Rowe *et al.* 2020).

Current limitations in the study and understanding of the role of microbiomes

Despite the growing interest, several limitations impede our comprehensive understanding of the intricate relationship between the microbiome and reproductive health.

One major limitation is the vast variability and complexity of the microbiome across individuals, tissues, and time (Bharti & Grimm 2021). For instance, the composition and function of the human microbiome is readily influenced by several factors such as age, sex, genetics, geography, lifestyle, and environmental exposures. This heterogeneity poses challenges in establishing consistent associations between specific microbial profiles and reproductive outcomes.

Another limitation stems from the lack of standardized protocols for sampling and analysis of the microbiome in reproductive biology (Bharti & Grimm 2021). Variations in sample collection techniques, storage, DNA extraction, sequencing methods, and bioinformatics pipelines can introduce biases and hinder comparability across studies. Developing standardized procedures and establishing robust quality control measures are essential to facilitate data harmonization and enable more accurate comparisons and meta-analyses (Kim *et al.* 2017).

Establishing causality between specific microbiome alterations and reproductive outcomes remains challenging. Many studies rely on correlation analysis which alone cannot determine whether microbial changes directly impact fertility or if they are merely bystanders given the bidirectional relationships of hormones and the microbiome. Functional studies using animal models, *in vitro* experiments, and intervention trials are necessary to unravel the mechanistic pathways and causal relationships underlying microbiome–reproduction interactions. Additionally, identifying specific microbial metabolites and their effects on reproductive tissues can provide valuable insights into microbial mechanisms of action.

Most of the current research focuses on cross-sectional studies, limiting our understanding of the temporal dynamics, volatility, and stability of the microbiome in relation to reproductive health (Park & Plantinga 2023). Longitudinal studies tracking microbiome changes over time considering baseline differences as well as influencing factors are crucial to capture the dynamic nature of microbial communities and assess how fluctuations impact reproductive outcomes. These studies would provide insights into the role of microbiome stability and resilience in maintaining fertility and optimizing reproductive interventions. Ethical considerations also pose limitations on conducting microbiome studies in reproductive health. Collecting samples from reproductive tissues, such as the uterus or fallopian tubes, are invasive and challenging. Additionally, designing studies that account for confounding factors, such as lifestyle, medications, hormonal fluctuations, and fertility treatments, requires careful consideration. Ethical guidelines and innovative study designs that overcome these challenges are essential to advance our knowledge in this field.

Future directions

Future studies should aim to comprehensively characterize the composition and dynamics of reproductive tract microbiomes across different species. Investigating variations in microbial populations, their interactions, and their responses to environmental factors could also provide valuable insights into the factors influencing fertility and reproductive health. A deeper understanding of the intricate interactions

between reproductive tract microbiomes and their host organisms is crucial. Elucidating the mechanisms by which microbiota influence reproductive processes, such as hormone regulation, gamete development, and implantation, can pave the way for targeted interventions to enhance or even suppress fertility.

The role of the microbiome in reproductive disorders and pathologies requires further exploration. Research should focus on discerning how dysbiosis, i.e. alterations in microbiome composition, may contribute to conditions such as endometriosis, polycystic ovary syndrome, and male infertility. Identifying specific microbial signatures associated with these pathologies can aid in early detection, prevention, and personalized treatment strategies. The potential therapeutic applications of microbiome modulation in reproductive health merits extensive investigation. Developing microbiome-based interventions, such as precision probiotics, prebiotics, synbiotics, and postbiotics, could offer novel approaches to enhance fertility outcomes, mitigate reproductive disorders, and improve assisted reproductive technologies success rates.

Considering the influence of environmental factors on reproductive tract microbiomes is essential. Studies should examine how lifestyle choices, dietary patterns, exposure to toxins, and antibiotic use impact microbiome composition and function. Such knowledge can guide the development of evidence-based recommendations to optimize reproductive health in humans as well as in animal species.

Comparative studies across different animal species can provide valuable insights into the evolution and conservation of microbiome-mediated reproductive processes. Investigating similarities and differences in reproductive tract microbiomes can shed light on fundamental mechanisms and highlight potential targets for therapeutic interventions in both humans and animals.

The integration of technologies to identify microbes and analyze their functions (including metagenomics, meta-transcriptomics, metabolomics, and mass spectrometry) will advance our understanding of the complex relationships between reproductive tract microbiomes and host physiology. These approaches can unveil functional pathways, microbial metabolites, and gene expression profiles that underpin reproductive processes, offering new avenues for therapeutic interventions.

Conclusions

Although the study of the microbiome in reproduction and fertility holds great promise, important limitations need to be addressed. Standardizing methodologies, establishing causality, conducting longitudinal studies, and navigating ethical considerations are crucial steps to overcome these limitations. There also is an urgent need to identify and classify a lot of unknown microbes. Advances in these areas will foster a more comprehensive understanding of the intricate interactions between microbiomes and reproductive health, ultimately leading to innovative interventions and personalized approaches to optimize fertility and reproductive outcomes.

Declaration of interest

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Author contribution statement

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