A Retrospective Narrative Mini-Review Regarding the Seminal Microbiota in Infertile Male

Bogdan Doroftei 1,2,3, Ovidiu-Dumitru Ilie 4,*, Ana-Maria Dabuleanu 1,2,3, Delia Hutanu 5 and Constantin-Cristian Vaduva 6,7,8

1 Faculty of Medicine, University of Medicine and Pharmacy “Grigore T. Popa”, University Street, No. 16, 700115 Iasi, Romania
2 Clinical Hospital of Obstetrics and Gynecology “Cuza Voda”, Cuza Voda Street, No. 34, 700038 Iasi, Romania
3 Origyn Fertility Center, Palace Street, No. 3C, 700032 Iasi, Romania
4 Department of Biology, Faculty of Biology, “Alexandru Ioan Cuza” University, Carol I Avenue, No. 20A, 700505 Iasi, Romania
5 Department of Biology, Faculty of Chemistry-Biology-Geography, West University of Timisoara, Vasile Parvan Avenue, No. 4, 300115 Timisoara, Romania
6 Department of Mother and Child Medicine, Faculty of Medicine, University of Medicine and Pharmacy, Petru Rares Street, No. 2, 200349 Craiova, Romania
7 Department of Obstetrics and Gynecology, Clinical Hospital Filantropia, Filantropia Street, No. 1, 200143 Craiova, Romania
8 Department of Infertility and IVF, HitMed Medical Center, Stefan cel Mare Street, No. 23-23A, 200130 Craiova, Romania

* Correspondence: ovidiuilie90@yahoo.com

Abstract: Background: Infertility is a global burden that affects both sexes with the male component remaining as an explored yet crucial research field that might offer novel evidence. Material and Methods: The present narrative mini-review aims to summarize all existing literature regarding the composition of the seminal microflora in infertile men. We performed searches in PubMed/Medline, ISI Web of Knowledge, Scopus, and ScienceDirect between 2018 and 2022 using a combination of keywords. Results: A total of n = 33 studies met the eligibility criteria and were further considered. From this, n = 14 were conducted on human patients, n = 3 on zebrafish (Danio rerio), n = 5 on rats, and n = 11 on mice. In twenty-five out of thirty-three papers, the authors sequenced the 16S rRNA; situations occurred where researchers focused on standard laboratory protocols. Lactobacillus and Bifidobacterium are widely recognized as putative beneficial lactic bacteria. These two entities are capable of restoring the host’s eubiosis to some extent, blocking pathogens’ proliferation and endotoxins, and even alleviating specific patterns encountered in disease(s) (e.g., obesity, type 1 diabetes) due to prolonged exposure to toxicants in adults or from a developmental stage. Over the years, distinct approaches have been perfected, such as the transfer of feces between two species or conventional rudimentary products with proven efficiency. Conclusions: The seminal microflora is decisive and able to modulate psychological and physiological responses. Each individual possesses a personalized microbial profile further shaped by exogenous factors, regardless of sex and species.

Keywords: semen; sperm; seminal fluid; spermatozoa; microflora; male infertility

1. Introduction

According to the American Society for Reproductive Medicine (ASRM), infertility designates the couple’s incapacity to conceive, carry, or deliver a baby following twelve months of regular unprotected intercourse [1].

Based on the latest reports and figures concerning the actual prevalence, the overall estimation reaches 13%, up to 15% worldwide. Of the total number, 30–40% of cases are attributable to both sexes, while roughly 20% is solely due to the male component [2–4] and for around fifty million couples, which reflect approximately 15%, to genitourinary
tract infections [5]. On the other hand, these percentages may be misleading since less than 2% of all known bacteria strains were successfully cultured and identified through conventional protocols [6].

However, numerous factors are responsible for male genital-tract inflammation, including prostatitis and/or epididymitis [7] in the case of acute or chronic infections related to infertility [8]. Thus, a pro-inflammatory cascade may trigger reactions in the chain reflected by low sperm quality via distinct mechanisms such as exacerbated oxidative stress (OS), hampered accessory gland secretion, anatomical sperm tract obstruction, or direct attack upon sperm by microorganisms [9].

Almost every constitutive site of the human body is colonized by microscopic entities; it is understandable that microorganisms may systematically impair the optimal parameters. Even their simple presence in semen samples may compromise the quality of sperm, with those responsible for contaminations originating from the urinary tract of infected patients during sexual intercourse [10].

Thus, in vitro studies brought insight into how bacteria affected sperm function without implied reactive oxygen species (ROS) or inflammatory cytokines [11,12], but with participation in agglutination of motile sperm, apoptosis, generation of immobilization factors, disturbance of acrosome reaction, and DNA fragmentation [13–19].

Noteworthy is the topic involving antibiotics regime, which caused controversy on whether pathogens are responsible for semen parameters abnormalities in vivo and how treatment presumably leads to an improvement [20]. For example, *Escherichia coli* is the most isolated microorganism identified and is known to adhere directly to the spermatozoa or synthesizing agents that consequently influence the reproductive potential [21,22].

These aspects discussed above fuel the interest to deepen this spectrum as a branch of research with substantial potential. Therefore, the present narrative mini-review aims to bring together the latest research by offering an updated overview, focusing on the underexplored direction of experimental models coupled with the limited knowledge and experience reported on human patients.

2. Methodology

This retrospective narrative mini-review follows the standard procedures established by Green et al. [23].

2.1. Database Search Strategy

The database used until inception (April 2022) was PubMed/Medline, ISI Web of Knowledge, Scopus, and ScienceDirect. The combination of keywords contains “microflora” and/or “microbiota” associated with “male infertility” and “semen”, “sperm” “seminal fluid”, and “spermatozoa”.

The adopted PubMed string was: male microbiota[Title/Abstract] OR male microflora[Title/Abstract] OR male infertility[Title/Abstract] AND semen[Title/Abstract] AND sperm [Title/Abstract] AND seminal fluid[Title/Abstract] AND spermatozoa[Title/Abstract].

2.2. Inclusion Criteria

Only articles written in English that enrolled human patients and experimental models (mice, rats, and zebrafish (*Danio rerio*)), respectively, conducted between 2018 and 2022 were considered.

2.3. Exclusion Criteria

Case report(s)/series, meta-analyses, review(s), standard or systematic, letters to the editor, conference posters, work protocols, and preprints were not considered suitable.

2.4. Study Selection

Four independent authors (B.D., O.-D.I., A.-M.D., and D.H.) screened the titles and abstracts of the retrieved results. We completed the assignment of relevant manuscripts
based on title, abstract, and full content. Any discrepancy was solved by consent with the remaining author (C.-C.V.).

2.5. Limitations of the Study

We concentrated on performing a narrative mini-review rather than a quantitative meta-analysis. This decision derives from data scarcity on this topic.

3. Results

A total of \( n = 1127 \) entries were returned during the established interval, from which \( n = 320 \) studies were conducted on human patients, \( n = 44 \) on zebrafish (Danio rerio), \( n = 431 \) on mice, and \( n = 332 \) on rats.

After we completed the assignment of all studies that initially met the eligibility criteria, we created a time series (2018–2022) in Microsoft Excel\textsuperscript{®} that contains the articles per year of publication, number, and database searched. Except for PubMed/Medline where we applied the aforementioned strategy, we restricted the searches to strictly research articles in English for ISI Web of Knowledge, Scopus, and ScienceDirect. Subsequently, we took each article and removed duplicates and foreign articles, letting only those that had as their main objective to demonstrate microbial translocations in the semen/seminal fluid/spermatozoa/sperm of humans, mice, rats, and zebrafish (Danio rerio).

According to the database search and the combination of keywords employed, the results are the following: \( n = 69 \) (male + microbiota + semen + infertility), \( n = 30 \) (male + microflora + semen + infertility), \( n = 91 \) (male + microbiota + sperm + infertility), \( n = 34 \) (male + microflora + sperm + infertility), \( n = 17 \) (male + microbiota + seminal fluid + infertility), \( n = 12 \) (male + microflora + seminal fluid + infertility), \( n = 44 \) (male + microbiota + spermatozoa + infertility), and \( n = 23 \) (male + microflora + spermatozoa + infertility). The same technique was applied for the experimental models as well with the mention that “male” was replaced with “zebrafish”, “Danio rerio”, “mice”, and “rats”, while “infertility” was removed from the search to increase the chances of covering as many results as possible. Therefore, the situation per each combination of keywords was as follows: \( n = 5 \) (Danio rerio + microbiota + semen), \( n = 12 \) (Danio rerio + microbiota + sperm), \( n = 0 \) (Danio rerio + microflora + semen), \( n = 7 \) (Danio rerio + microflora + seminal fluid), \( n = 4 \) (zebrafish + microflora + semen), \( n = 8 \) (zebrafish + microflora + sperm), \( n = 3 \) (zebrafish + microflora + seminal fluid), and \( n = 5 \) (zebrafish + microflora + spermatozoa). For rodent models, we found the following: \( n = 93 \) (mice + microbiota + semen), \( n = 156 \) (mice + microbiota + sperm), \( n = 29 \) (mice + microflora + seminal fluid), \( n = 49 \) (mice + microflora + spermatozoa), \( n = 32 \) (mice + microflora + semen), \( n = 47 \) (mice + microflora + sperm), \( n = 7 \) (mice + microflora + seminal fluid), and \( n = 18 \) (mice + microflora + spermatozoa) on mice, and \( n = 91 \) (rats + microbiota + semen), \( n = 88 \) (rats + microbiota + sperm), \( n = 17 \) (rats + microflora + seminal fluid), \( n = 33 \) (rats + microflora + spermatozoa), \( n = 42 \) (rats + microflora + semen), \( n = 35 \) (rats + microflora + sperm), \( n = 9 \) (rats + microflora + seminal fluid), and \( n = 17 \) (rats + microflora + spermatozoa). Chronologically, the number of studies published per year is the following: \( n = 37 \) in 2018, \( n = 45 \) in 2019, \( n = 75 \) in 2020, \( n = 104 \) in 2021, and \( n = 59 \) in 2022 in human patients; \( n = 1 \) in 2018, \( n = 6 \) in 2019, \( n = 5 \) in 2020, \( n = 21 \) in 2021, and \( n = 11 \) in 2022 in zebrafish (Danio rerio); \( n = 25 \) in 2018, \( n = 71 \) in 2019, \( n = 92 \) in 2020, \( n = 124 \) in 2021, and \( n = 119 \) in 2022 in mice; \( n = 40 \) in 2018, \( n = 44 \) in 2019, \( n = 56 \) in 2020, \( n = 113 \) in 2021, and \( n = 79 \) in 2022 in rats. According to the database and subjects (humans, zebrafish, mice, and rats), the number of studies published in this context were: \( n = 40 \), \( n = 3 \), \( n = 41 \), and \( n = 19 \) in PubMed/Medline; \( n = 34 \), \( n = 3 \), \( n = 27 \), and \( n = 18 \) in ISI Web of Knowledge; \( n = 84 \), \( n = 4 \), \( n = 28 \), and \( n = 15 \) in Scopus; and \( n = 162 \), \( n = 34 \), \( n = 335 \), and \( n = 280 \) in ScienceDirect, respectively. After removing duplicates and studies written in foreign languages, \( n = 33 \) were considered further. From these studies, \( n = 14 \) were conducted on human patients, \( n = 3 \) on zebrafish (Danio rerio), \( n = 5 \) on rats, and \( n = 11 \) on mice.
3.1. Seminal Microflora Analysis of the 16S rRNA through Next-Generation Sequencing

It has been revealed by Monteiro et al. [11] that among all semen samples analyzed, *Enterococcus* was dominant to the detriment of *Lactobacillus*, a scenario that represented 0.5% of all entities discovered. Moreover, *Neisseria, Pseudomonas,* and *Klebsiella* increment coupled with lactic acid bacteria depletion is related to oligoasthenoteratozoospermia and seminal hyperviscosity. The discoveries of Alfano et al. [24] are of interest, demonstrating the increase in DNA bacterial amount in contrast with the spermatogenesis of other examined group individuals. *Actinobacteria* and *Firmicutes* were predominant, but the authors highlighted a decrease in richness and diversity in patients with complete germine cell aplasia. This evidence reflects the predominance of *Actinobacteria* and the absence of *Clostridia*. This might further indicate an aging phenomenon of the testes. Testes present tissue-associated symbiotic bacteria-harboring *Actinomycetes*, *Bacteroides*, *Pachybacteria*, and *Proteus* in seminoma normozoospermic men and *Actinomycetes* and *Sclerotinia* in non-obstructive azoospermia. Even though testicular sperm microbiota is low in biomass, it is abundant in contamination (50%-70%). There is significance in genera belonging to *Blautia, Cellulosibacter, Clostridium XIVa, Clostridium XIVb, Clostridium XVIII, Collinsella, Prevotella, Prolixibacter, Robinsonella,* and *Wandonia* in immature spermatozoa, according to Molina et al. [25].

The semen within normal parameters contains elevated amounts of *Lactobacillus* and exerts a potent effect on its parameters, accompanied by a balanced ratio of *Prevotella, Streptococcus,* and *Staphylococcus* genera [26]. However, there is controversy surrounding this topic. Yang et al. [27] uncovered an abundance of *Lactobacillus* in oligoasthenospermic compared to their counterparts. *Prevotella* was associated with low-quality semen. The most numerous associations in dispermic individuals were *Pseudomonas, Prevotella* and *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes,* and *Fusobacteria. Serratia, Acinetobacter, Pseudomonas, Escherichia,* and *Stenotrophomonas* interfere within current methodologies. These microorganisms systematically impact the semen morphology and deoxyribonucleic acid, even leading to mitochondrial disruption.

Almost every site of the human body is populated. *Proteobacteria* and *Corynebacterium* species are negatively associated with embryos through in vitro fertilization (IVF). Štšepetova et al. [28] considered the presence of *Staphylococcus* species, *Alphaproteobacteria* and *Enterobacteriaceae,* as indicators of embryo and sperm quality. Environmental factors also change the bacterial ratio of body fluids. Yao et al. [29] found that *Staphylococcus, Corynebacterium,* and *Corynebacterium_1* genus were present in almost all samples analyzed. The existence of distinct signatures in *α*- and *β*-diversity between semen and rectal samples was provided by Lundy et al. [30]. It marks an increase in *Aerococcus* in urine and semen and a decrease in *Collinsella* in the semen of infertile men. The seminal fluid bacterial concentration is lower, but the composition is higher in diversity. There are discrepancies between categories, where azoospermic men have a relative abundance of *Mycoplasma* and *Ureaplasma* compared with *Lactobacillus* in normospermic semen and corresponding vaginal samples. *Gardnerella* is also a part of the microflora in both sexes, whereas *Prevotella* is found solely in women [31]. In Table 1, a summarization of microbiota changes in human patients can be found.
Table 1. Summarization of the studies in which the authors sequenced the 16S rRNA in human individuals.

| Number of Participants | Hypervariable Region | Sequencer     | Microbial Changes                                                                 | Reference |
|------------------------|----------------------|---------------|-----------------------------------------------------------------------------------|-----------|
| n = 118 participants   | V3–V6                | Ion PGM       | Proteobacteria ↑                    | [11]      |
|                        |                      |               | Neisseria ↑                         |           |
|                        |                      |               | Klebsiella ↑                         |           |
|                        |                      |               | Pseudomonas ↑                        |           |
|                        |                      |               | Lactobacillus ↓                      |           |
| n = 15 participants    | V3–V5                | 454-GS Junior | Actinobacteria ↓↑                    | [24]      |
|                        |                      |               | Bacteroides ↓                        |           |
|                        |                      |               | Firmicutes ↓                         |           |
|                        |                      |               | Proteobacteria ↓                     |           |
|                        |                      |               | Clostridia ↓                         |           |
|                        |                      |               | Peptoniphilus asaccharolyticus ↓     |           |
| n = 94 participants    | V1–V2                | MiSeq         | Actinobacteria ↑                    | [26]      |
|                        |                      |               | Bacteroides ↑                        |           |
|                        |                      |               | Firmicutes ↑                         |           |
|                        |                      |               | Proteobacteria ↑                     |           |
| n = 10 participants    | V3–V4                | MiSeq         | Staphylococcus ↑                    | [29]      |
|                        |                      |               | Corynebacterium ↑                   |           |
|                        |                      |               | Corynebacterium_1 ↑                  |           |
| n = 159 participants   | V1–V2                | HiSeq 2500    | Ureaplasma ↑                        | [27]      |
|                        |                      |               | Bacteroides ↑                        |           |
|                        |                      |               | Anaerococcus ↑                       |           |
|                        |                      |               | Finegoldia ↑                         |           |
|                        |                      |               | Lactobacillus ↑                      |           |
|                        |                      |               | Acinetobacter lwoffii ↑              |           |
| n = 50 couples         | V2–V3                | 454 FLX       | Lactobacillus ↑                      | [28]      |
|                        |                      |               | Alphaproteobacteria ↑                |           |
|                        |                      |               | Bacteroides ↓                        |           |
|                        |                      |               | Alphaproteobacteria ↓                |           |
| n = 37 participants    | V3–V4                | MiSeq         | Aerococcus ↑                         | [30]      |
|                        |                      |               | Anaerococcus ↓                       |           |
|                        |                      |               | Prevotella ↑                         |           |
|                        |                      |               | Pseudomonas ↑                        |           |
| n = 11 participants    | V3–V4                | MiSeq         | Blautia ↑                            | [25]      |
|                        |                      |               | Cellulosibacter ↑                    |           |
|                        |                      |               | Clostridium XIVa ↑                   |           |
|                        |                      |               | Clostridium XIVb ↑                   |           |
|                        |                      |               | Clostridium XVIII ↑                  |           |
|                        |                      |               | Collinsella ↑                        |           |
|                        |                      |               | Prevotella ↑                         |           |
|                        |                      |               | Prolivibacter ↑                      |           |
|                        |                      |               | Robinsoniella ↑                      |           |
|                        |                      |               | Wandonia ↑                           |           |
| n = 36 couples         | V4                   | MiSeq         | Mycoplasma ↑                         | [31]      |
|                        |                      |               | Ureaplasma ↑                         |           |
|                        |                      |               | Lactobacillus ↑                      |           |
|                        |                      |               | Gardnerella ↑                        |           |
|                        |                      |               | Lactobacillus jensenii ↑             |           |
|                        |                      |               | Faecalibacterium ↑                   |           |
|                        |                      |               | Proteobacteria ↓                     |           |
|                        |                      |               | Prevotella ↓                         |           |
|                        |                      |               | Bacteroides ↓                        |           |
|                        |                      |               | Firmicutes/Bacteroides ratio ↓       |           |

↑—increase; ↓—decrease; ↑↓—variations rely on the study design/patient allocations.
3.2. Seminal Microflora Analysis through Standard Laboratory Analyses

Mändar et al. [32] revealed a positive correlation between those with non-sexual and sexual experiences in terms of bacterial concentration and diversity, further dependent on the enrichment of the seminal fluid. Vorobets et al. [33] discovered that *Ureaplasma parvum* and *Ureaplasma urealyticum* caused infections of the genitourinary tract system, identifying *Enterococcus faecalis* known for metabolizing active compounds constitutive of specific drugs [34]. Another marker besides those mentioned above of hormonal impairments after possible fertility dysfunction might be the presence of *Lactobacillus* species in the ejaculate [35]. A possible dysbacteriosis may disturb the integrity of commensal microorganisms, a case when a proliferation of opportunistic pathogens might occur. In this case, even sexually transmitted microorganism balance reported in asymptomatic men could be perturbed, generating a cascade effect on sexual and reproductive health. DNA from *Pseudomonas aeruginosa*, *Chlamydia trachomatis*, *Staphylococcus aureus* and epidermidis, *Klebsiella pneumoniae*, *Neisseria gonorrhoeae*, species of *Lactobacillus*, and *Escherichia coli* were dominant and reported at different values [36].

3.3. Seminal Microflora Analysis on Experimental Models

3.3.1. Zebrafish (*Danio rerio*)

Numerous factors influence fertility status, including obesity and chemical compound usage on a large scale. Thus, Su et al. [37] successfully obtained a zebrafish obesity model following egg yolk powder administration. Besides the testicular inflammation, increased pathogenic bacteria proliferation in obese *Danio rerio* was observed. These observations were consistent with the study of Jiang et al. [38], where they tested the single and joint effects of tebuconazole (TEB) and difenoconazole (DIF). A mixture of TEB and DIF displayed additive effects on the acute toxicity but was less pronounced than TEB and DIF alone on the liver, gonad, and intestinal microflora. Valcarce et al. [39] have already shown that a feeding regime based on probiotics may be a time-cost-efficient approach to rescuing low fertility status. The group that received *Lactobacillus rhamnosus* CECT8361 and *Bifidobacterium longum* CECT7347 (1:1) for three weeks had the same weight but improved sperm parameters. In Table 2, a summarization of microbiota changes in *Danio rerio* can be found.

Table 2. Summarization of the studies in which the authors sequenced the 16S rRNA in zebrafish.

| Hypervariable Regions | Sequencer | Microbiota Changes | Reference |
|-----------------------|-----------|--------------------|-----------|
| V3–V4                 | Miseq PE300 | *Lactobacillus* ↑  
*Bifidobacterium* ↓  
*Proteobacteria* ↑  
*Firmicutes* ↑  
*Actinobacteria* ↓  
*Escherichia-Shigella* ↑ | [37] |
| V3–V4                 | Miseq PE300 | *Proteobacteria* ↑  
*Firmicutes* ↑  
*Bacteroidetes* ↑  
*Fusobacteria* ↓ | [38] |

↑—increase; ↓—decrease.

3.3.2. Rats

It has been recently postulated by Wang et al. [40] that Di-(2-ethylhexyl)phthalate (DEHP) toxicity is related to the model used and strains’ changes in the gut. Sprague-Dawley rats were the most vulnerable to bacterial translocations from all four groups: Wistar rats, BALC/C, and C57BL/6j mice. Liu et al. [41] reported an association between gut alteration and defective spermatogenesis, consistent with data obtained by Zhang et al. [42] following the exposure to glyphosate (GLY) and phthalate dibutyl phthalate (DBP). DBP causes seminiferous atrophy and spermatogenic cell apoptosis. A joint effect of fluoride and arsenic reduces testicular weight and hormone levels. Liu et al. [43] showed a positive
association with cells’ natural degradation process. The vaginal microbiota probiotic *Lactobacillus crispatus* impacts sperm activity. Li et al. [44] acknowledged that *Lactobacillus crispatus* is responsible for low-number-related pregnancies due to its adhesion property and even accounts for some unexplained infertility. In Table 3, a summarization of microbiota changes in rats can be found.

**Table 3.** Summarization of the studies in which the authors sequenced the 16S rRNA in rats.

| Model                        | Hypervariable Regions | Sequencer          | Microbiota Changes                                      | Reference |
|------------------------------|-----------------------|--------------------|----------------------------------------------------------|-----------|
| Wistar, Sprague-Dawley       | V3–V4                 | MiSeq PE300        | Proteobacteria ↑                                          | [40]      |
|                              |                       |                    | Firmicutes ↑                                              |           |
|                              |                       |                    | Firmicutes/Bacteroidetes ratio ↑                          |           |
|                              |                       |                    | Oscillospira ↑                                            |           |
|                              |                       |                    | Peptostreptococcaceae ↑                                   |           |
|                              |                       |                    | Mycoplasma ↑                                              |           |
|                              |                       |                    | Roseburia ↑                                               |           |
|                              |                       |                    | Clostridiales ↑                                           |           |
|                              |                       |                    | Sutterella ↑                                              |           |
|                              |                       |                    | Clostridiales ↑                                           |           |
|                              |                       |                    | RF32 ↑                                                   |           |
|                              |                       |                    | Christensenellaceae ↑                                     |           |
|                              |                       |                    | Blautia ↑                                                 |           |
|                              |                       |                    | rc4–4 ↑                                                   |           |
|                              |                       |                    | Prevotella ↓                                              |           |
|                              |                       |                    | Actinomyces ↑                                             |           |
|                              |                       |                    | Arthrobacter ↑                                            |           |
|                              |                       |                    | Porphyromonas ↑                                           |           |
|                              |                       |                    | Bacteroides ↓                                             |           |
| Sprague-Dawley               | V3–V4                 | MiSeq              | Bacteroidetes ↑                                           | [41]      |
|                              |                       |                    | Prevotella_1 ↑                                            |           |
| Sprague-Dawley               | V4                    | HiSeq 2500         | Bacteroidetes ↑                                           | [42]      |
|                              |                       |                    | Prevotella ↑                                              |           |
|                              |                       |                    | Prevotella copri ↑                                        |           |
| Sprague-Dawley               | 338F–806R primers     | -                  | SMB53 ↑↑                                                 |           |
|                              |                       |                    | p-75-a5 ↑↑                                                |           |
|                              |                       |                    | rc4–4 ↑                                                   |           |
|                              |                       |                    | Phascolarctobacterium ↑                                   |           |
|                              |                       |                    | Veillonella ↑                                             |           |
|                              |                       |                    | Anaerostipes ↑                                            |           |
|                              |                       |                    | Desulfovibrio ↓                                           |           |
|                              |                       |                    | Corynebacterium ↓                                         |           |
|                              |                       |                    | Trichococcus ↑                                            |           |
|                              |                       |                    | Lachnobacterium ↑                                         |           |
|                              |                       |                    | Epulopiscium ↑                                            |           |
|                              |                       |                    | Allobaculum ↓                                             |           |

↑—increase; ↓—decrease; ↑↓—variations rely on the study design/patient allocations.

3.3.3. Mice

A high-fat diet (HFD) for prolonged periods of time ultimately leads to metabolic disorders and impaired sperm production in males [45,46], associated with high circulating endotoxins and decreased spermatogenesis. Conventional dietary products mainly target obese-induces dysbacteriosis [47,48] following the administration of *Lactobacillus fermentum* NCDC 400 and *Lactobacillus rhamnosus* NCDC 610 or type 1 diabetes [49,50] through fecal microbiota transplantation (FMT) [51]. Moreover, it has been demonstrated on previous occasions that gut flora, semen parameters, and testosterone deficiency might be influenced in both ways by probiotics *Lactobacillus plantarum* TW1-1 [52], alginate oligosaccharides [53],
or selenium [54] and doxycycline [55]. In Table 4, a summarization of microbiota changes in mice can be found.

Table 4. Summarization of the studies in which the authors sequenced the 16S rRNA in mice.

| Model          | Hypervariable Region | Sequencer       | Microbiota Changes | Reference |
|----------------|----------------------|-----------------|--------------------|-----------|
| C57BL/6        | V3–V4                | HiSeq 4000      | Bacteroidetes ↓ Verrucomicrobia ↓ Firmicutes ↑ Proteobacteria ↑ | [45]      |
| C57BL/6J       | V4                   | MiSeq           | Corynebacterium ↑ Rikenellaceae ↑ | [46]      |
| C57BL/6J       | V3–V4                | HiSeq 6000      | Verrucomicrobiace ↑ Gammaproteobacteria ↑ Mollicutes ↑ Bacteroidia ↓ Betaproteobacteria ↓ | [47]      |
| KK-Ay C57BL/6J | V3–V4                | MiSeq           | Weissella confusa ↓ Clostridium sp. ND2 ↓ Anaerotruncus colihominis DSM 17,241 ↓ [Clostridium] leptum ↓ | [49]      |
| KK-Ay C57BL/6  | V3–V4                | MiSeq           | Bacteroidales S24-7 group ↑ Bifidobacterium ↑ Akkermansia ↑ | [50]      |
| CD-1           | V3–V4                | HiSeqTM 2500    | Lactobacillus ↑    | [51]      |
| C57BL/6        | V4                   | MiSeq           | Bacteroidetes ↓ Firmicutes ↑ Deferribacteres ↑ | [52]      |
| ICR            | V3–V4                | HiSeq X Ten     | Lactobacillaceae ↑ Desulfovibrionaceae ↓ Proteobacteria ↑ Bacteroidales ↑ | [53]      |
| BALB/c         | V3–V4                | MiSeq           | Lachnospiraceae ↑ Ruminococcaceae ↑ Christensenellaceae ↑ Lactobacillus ↑ | [54]      |
| C57BL/6J       | V3–V4                | MiSeq           | Candidatus ↑ Saccharimonas ↓ Ruminococcus I ↓ Helicobacter ↓ Anaeroplasma ↓ | [55]      |

↑—increase; ↓—decrease; ↑↓—variations rely on the study design/patient allocations.

3.4. Potential Therapeutic Approaches

According to the World Health Organization (WHO), probiotics are living microorganisms derived mainly from cultured dairy products. On the other hand, prebiotics is committed to inducing the growth or activity of beneficial microorganisms. Presently, probiotics are the most powerful and safe alternative in re-establishing the host’s eubiosis with proven efficiency in almost every field of expertise when applied. There is currently one randomized controlled trial underway titled “Effect of Antioxidant Probiotic Administration on Seminal Quality and Reproductive Outcomes,” in the recruitment stage and expected for completion in May 2023 (NCT04585984). The estimated number of participants is n = 280 but limited to a single center. Briefly, the authors aim to allocate individuals into two equal
groups (n = 140) and subsequently administer a mixture of Lactobacillus rhamnosus and Bifidobacterium longum for three weeks.

Retrospectively, Barbonetti et al. [56] discussed their preliminary data according to which probiotics combination of Lactobacillus brevis (CD2), Lactobacillus salivarius (FV2), and Lactobacillus plantarum (FV9) confer protection of human spermatozoa from radical oxygen species in case of a vaginal disease, thereby improving the chances of fertilization potential. Various studies subsequently emphasized the role of probiotics in improving fertilization potential and related endocrine and sperm parameters. As previously indicated by Valcarce et al. [57], a 3-week regime with Lactobacillus rhamnosus CECT8361 and Bifidobacterium longum CECT7347 significantly enhances sperm quality in asthenozoospermic males, followed by a notable decrease of DNA fragments and intracellular H$_2$O$_2$.

Moreover, during a 6-month diet with Lactobacillus paracasei B21060 (Flortec) administration in infertile men, the authors also observed a refinement in the volume of the ejaculate, sperm concentration, progressive motility, and the percentage of typical forms as indicated by Maretti et al. [58]. The same team further noted an improvement in hormone levels compared with the control group after the Flortec regime [58]. The same parameters were investigated, excepting sperm count, live sperm, and serum and seminal total antioxidant capacity. Helli et al. [59] revealed a decrease in plasma pro-inflammatory, serum, and seminal malondialdehyde after a diet containing Lactobacillus and Bifidobacteria species. This strategy was successfully translated to murine models as well, among the most preferred species to study reproductive potential, including Lactobacillus coagulans/casei/rhamnosus PB01 (DSM 14870), Lactobacillus and Bifidobacterium, and occasionally Candida utilis (Cu. M02) and Streptococcus thermophilus (St. S07) [59–63]. Lactobacillus rhamnosus CECT8361 and Bifidobacterium longum CECT7347 had a beneficial role in humans and rodents, as suggested by Valcarce et al. [39]. Separate lines of evidence proved the same in the case of prebiotics. They increased the Lactobacillus and Bifidobacterium ratio, thereby elevating short-chain fatty acids essential to regulate metabolic and immune function [64,65]. For example, Rodrigues et al. [66] found that oligofructose supplementation in the diet of mice promoted an alteration in steroidogenesis that subsequently affected plasma corticosterone and testosterone levels.

Moreover, another alternative to alleviate dysbiosis involves the installation of processed stool, obtained from a healthy individual, in a patient’s small intestine. Thus, FMT became a so-called method of choice, where Zhang et al. [67] even demonstrated how FMT leads to male fertility ameliorations. This technique induced significant shifts of beneficial bacteria such as Bacteroidetes, Bifidobacterium, Sphingomonas, and Campylobacter. Their presence was associated with the enhancement of spermatogenesis-related genes involving blood and testicular metabolome, spermatogenesis, and even the protein expression of glutathione peroxidase 1 (GPX1). Despite its benefits, the number of papers focused on FMT to treat male infertility in humans is absent from the current literature.

4. Conclusions

Compared to the existing evidence in the literature, this manuscript aimed to shed light on an already underexplored field of research found in its infancy stage by providing a different perspective that also involves experimental models. Concerning experiences involving human-related research, certain limitations, legal jurisdictions, and social and ethical values should be imperatively applied and overwhelmed, which is why we wanted to reflect the role of this branch by including mice, rats, and zebrafish (Danio rerio) since they are the promoters of a myriad of discoveries translated into the clinical practice.

Probiotics containing Lactobacillus and Bifidobacterium, which are putative beneficial microorganisms, may restore the eubiosis following the administration of a specific diet to mimic symptomatology. However, referring to the overall potential in countering the associated changes in obesity, type 1 diabetes, and acute toxicity due to prolonged exposure is yet to be explored. Moreover, recent studies contributed to the uprising trend of knowledge. Novel approaches include the transfer of fecal matter between two subjects, how conven-
tional products alleviate the symptoms, and the presence of microorganisms interfere with the pregnancy-related numbers. Starting from our rationale, we will soon contribute to an increasing trend of information regarding how probiotics impact reproductive status since the existing literature supports this kind of relationship.

However, it is hard to argue that distinct urogenital sites harbor a unique microbiome because of the difficulty to acquire exudates free of contamination. One possible explanation resides within the capacity of locally adapted communities for the predisposition of dispersal and colonization via sexual transmission.

Thus, from our point of view, we hope this mini-review may accentuate the scarcity of data and be further used as a support pillar to encourage research teams and increase interest in new large-scale studies that could respond to the plethora of questions regarding how microorganisms impact reproductive traits and success, regardless of sex, and arguably fill this gap in our understanding.

Author Contributions: B.D., O.-D.I., A.-M.D. and D.H. (Conceptualization, Data curation, Investigation, Formal analysis, Methodology, Writing—original draft); B.D. and C.-C.V. (Conceptualization, Methodology, Supervision, Validation, Project Administration, Writing—Review and Editing). All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss. Fertil. Steril. 2008, 90, S60. [CrossRef] [PubMed]
2. Agarwal, A.; Mulgund, A.; Hamada, A.; Chyatte, M.R. A unique view on male infertility around the globe. Reprod. Biol. Endocrinol. 2015, 13, 37. [CrossRef] [PubMed]
3. Inhorn, M.C.; Patrizio, P. Infertility around the globe: New thinking on gender, reproductive technologies and global movements in the 21st century. Hum. Reprod. Update 2015, 21, 411–426. [CrossRef] [PubMed]
4. Winters, B.R.; Walsh, T.J. The Epidemiology of Male Infertility. Urol. Clin. N. Am. 2015, 41, 37. [CrossRef] [PubMed]
5. Pellati, D.; Mylonakis, I.; Bertoloni, G.; Fiore, G.; Andrisani, A.; Ambrosini, G.; Armanini, D. Genital tract infections and infertility. Eur. J. Obstet. Gynecol. Reprod. Biol. 2008, 140, 3–11. [CrossRef]
6. Wade, W. Unculturable bacteria—The uncharacterized organisms that cause oral infections. J. R. Soc. Med. 2002, 95, 81–83.
7. Keck, C.; Gerber-Schäfer, C.; Clad, A.; Wilhelm, C.; Breckwoldt, M. Seminal tract infections: Impact on male fertility and treatment options. Hum. Reprod. Update 1998, 4, 891–903. [CrossRef]
8. Mándar, R.; Punab, M.; Korrovits, P.; Türk, S.; Ausmees, K.; Lapp, E.; Preem, J.-K.; Oopkaup, K.; Salumets, A.; Truu, J. Seminal microbiome in men with and without prostatitis. Int. J. Urol. 2017, 24, 211–216. [CrossRef]
9. Condorelli, R.A.; Russo, G.I.; Calogero, A.E.; Morgia, G.; La Vignera, S. Chronic prostatitis and its detrimental impact on sperm parameters: A systematic review and meta-analysis. J. Endocrinol. Investig. 2017, 40, 1209–1218. [CrossRef]
10. Purvis, K.; Christiansen, E. Infection in the male reproductive tract. Impact, diagnosis and treatment in relation to male infertility. Int. J. Androl. 1993, 16, 1–13. [CrossRef]
11. Monteiro, C.; Marques, P.I.; Cavadas, B.; Damião, I.; Almeida, V.; Barros, N.; Barros, A.; Carvalho, F.; Gomes, S.; Seixas, S. Characterization of microbiota in male infertility cases uncovers differences in seminal hyperturbidiscoidy and oligoastheno-tazoosperma possibly correlated with increased prevalence of infectious bacteria. Am. J. Reprod. Immunol. 2018, 79, e12838. [CrossRef] [PubMed]
12. Calogero, A.E.; Duca, Y.; Condorelli, R.A.; La Vignera, S. Male accessory gland inflammation, infertility, and sexual dysfunctions: A practical approach to diagnosis and therapy. Andrology 2017, 5, 1064–1072. [CrossRef] [PubMed]
13. Villegas, J.; Schulz, M.; Soto, L.; Sanchez, R. Bacteria induce expression of apoptosis in human spermatozoa. Apoptosis 2005, 10, 105–110. [CrossRef] [PubMed]
14. Kaur, S.; Prabha, V. Receptor mediated amelioration of the detrimental effects of sperm agglutinating factor on sperm parameters. Andrology 2013, 1, 624–631. [CrossRef] [PubMed]
15. Nunez-Calonge, R.; Caballero, P.; Redondo, C.; Baquero, F.; Martinez-Ferrer, M.; Meseguer, M.A. Ureaplasma urealyticum reduces motility and induces membrane alterations in human spermatozoa. *Hum. Reprod.* 1998, 13, 2756–2761. [CrossRef]

16. Boguen, R.; Treullen, F.; Uribe, P.; Villegas, J.V. Ability of *Escherichia coli* to produce hemolysis leads to a greater pathogenic effect on human sperm. *Fertil. Steril.* 2015, 103, 1155–1161. [CrossRef]

17. Ma, X.; Gao, X. The effect of *Ureaplasma urealyticum* on the level of P34H expression, the activity of hyaluronidase, and DNA fragmentation in human spermatozoa. *Am. J. Reprod. Immunol.* 2017, 77, e12600. [CrossRef]

18. La Vignera, S.; Vicari, E.; Condorelli, R.A.; D’Agata, R.; Calogero, A.E. Male accessory gland infection and sperm parameters (review). *Int. J. Androl.* 2011, 34, e330–e347. [CrossRef]

19. Merino, G.; Carranza-Lira, S.; Murrieta, S.; Rodriguez, L.; Cuevas, E.; Morán, C. Bacterial infection and semen characteristics in infertile men. *Arch. Androl.* 1995, 35, 43–47. [CrossRef]

20. Dohle, G.R.; Weidner, W.; Jungwirth, A.; Colpi, G.; Papp, G.; Pomerol, J.; Hargreve, T.B. *Guidelines on Male Infertility*; European Association of Urology: Arnhem, The Netherlands, 2004.

21. Diemer, T.; Huwe, P.; Ludwig, M.; Schroeder-Printzen, I.; Michelmann, H.W.; Schiefer, H.G.; Weidner, W. Influence of autogenous leukocytes and *Escherichia coli* on sperm parameters in vitro. *Andrologia* 2003, 35, 100–105. [CrossRef]

22. Diemer; Huwe; Michelmann; Mayer; Schiefer; Weidner. *Escherichia coli*-induced alterations of human spermatozoa. An electron microscopy analysis. *Int. J. Androl.* 2000, 23, 178–186. [CrossRef] [PubMed]

23. Green, B.N.; Johnson, C.D.; Adams, A. Writing narrative literature reviews for peer-reviewed journals: Secrets of the trade. *J. Chiropr. Med.* 2006, 5, 101–117. [CrossRef] [PubMed]

24. Alfano, M.; Ferrarese, R.; Locatelli, I.; Ventimiglia, E.; Ippolito, S.; Gallina, P.; Cesana, D.; Canducci, F.; Pagliardini, L.; Viganò, P.; et al. Testicular microbiome in azoospermic men—First evidence of the impact of an altered microenvironment. *Hum. Reprod.* 2018, 33, 1212–1217. [CrossRef] [PubMed]

25. Molina, N.M.; Plaza-Diaz, J.; Vilchez-Vargas, R.; Sola-Leyva, A.; Vargas, E.; Mendoza-Tesarik, R.; Galán-Lázaro, M.; de Guevara, N.M.L.; Tesarik, J.; Altmae, S. Assessing the testicular sperm microbiome: A low-biomass size with abundant contamination. *Reprod. Biomed. Online* 2021, 43, 523–531. [CrossRef]

26. Baud, D.; Pattaroni, C.; Vulliemoz, N.; Castella, V.; Marsland, B.J.; Stojanov, M. Sperm Microbiota and Its Impact on Semen Parameters. *Front. Microbiol.* 2019, 10, 234. [CrossRef]

27. Yang, H.; Zhang, J.; Xue, Z.; Zhao, C.; Lei, L.; Wen, Y.; Dong, Y.; Yang, J.; Zhang, L. Potential Pathogenic Bacteria in Seminal Microbiota of Patients with Different Types of Dyspermatism. *Sci. Rep.* 2020, 10, 6876. [CrossRef]

28. Stšepetova, J.; Baranova, J.; Simm, J.; Parm, Ü.; Rööp, T.; Sokmann, S.; Korrovits, P.; Jaagura, M.; Rosenstein, K.; Salumets, A.; et al. The complex microbiome from native semen to embryo culture environment in human in vitro fertilization procedure. *Reprod. Biol. Endocrinol.* 2020, 18, 3. [CrossRef]

29. Tao, T.; Han, X.; Guan, T.; Wang, Z.; Zhang, S.; Liu, C.; Liu, C.; Chen, L. Effect of indoor environmental exposure on seminal microbiota and its application in body fluid identification. *Forensic Sci. Int.* 2020, 314, 110417. [CrossRef]

30. Lundy, S.D.; Sangwan, N.; Parekh, N.V.; Selvam, M.K.P.; Gupta, S.; McCaffrey, P.; Bessoff, K.; Vala, A.; Agarwal, A.; Sabanegh, E.S.; et al. Functional and Taxonomic Dysbiosis of the Gut, Urine, and Semen Microbiomes in Male Infertility. *Eur. Urol.* 2021, 79, 826–836. [CrossRef]

31. Okweluogu, S.I.; Ikechebelu, J.I.; Agbakoba, N.R.; Anukam, K.C. Microbiome Compositions from Infertile Couples Seeking In Vitro Fertilization, Using 16S rRNA Gene Sequencing Methods: Any Correlation to Clinical Outcomes? *Front. Cell. Infect. Microbiol.* 2021, 11, 709372. [CrossRef]

32. Mândar, R.; Türk, S.; Korrovits, P.; Ausmees, K.; Punjab, M. Impact of sexual debut on culturable human seminal microbiota. *Andrology* 2018, 6, 510–512. [CrossRef] [PubMed]

33. Vorobets, M.Z.; Melnyk, O.V.; Kovalenko, I.V.; Fafula, R.V.; Borzhevsky, A.T.; Vorobets, Z.D. Condition of urogenital tract microbiotes and pro- and antioxidant system in male azoospermia. *Regul. Mech. Biosyst.* 2021, 12, 696–701. [CrossRef]

34. Maini Rekdal, V.; Bess, E.N.; Bisanz, J.E.; Turnbaugh, P.J.; Balskus, E.P. Discovery and inhibition of an interspecies gut bacterial pathway for Levodopa metabolism. *Science* 2019, 364, eaau6323. [CrossRef] [PubMed]

35. Pochernikov, D.G.; Postovoytenko, N.P.; Getman, V.V.; Galkina, I.S. Diagnostic significance of *Lactobacillus* spp. identification in ejaculate. *Bull. Russ. State Med Univ.* 2020, 3, 44. [CrossRef]

36. Rivera, V.V.; Cardona Maya, W.D.; Suárez, J.P. The relationship between sexually transmitted bacteria, microbiota and seminal quality in asymptomatic men. *Asian J. Urol.* 2021. [CrossRef]

37. Su, Y.; He, L.; Hu, Z.; Li, Y.; Zhang, Y.; Fan, Z.; Zhao, K.; Zhang, H.; Liu, C. Obesity Causes Abrupt Changes in the Testicular Microbiota and Sperm Motility of Zebrafish. *Front. Immunol.* 2021, 12, 639239. [CrossRef] [PubMed]

38. Jiang, J.; Chen, L.; Liu, X.; Wang, L.; Wu, S.; Zhao, X. Histology and multi-omic profiling reveal the mixture toxicity of tebuconazole and difenoconazole in adult zebrafish. *Sci. Total Environ.* 2021, 795, 148777. [CrossRef]

39. Valcarce, D.G.; Riesco, M.F.; Martínez-Vázquez, J.M.; Robles, V. Diet Supplemented with Antioxidant and Anti-Inflammatory Probiotics Improves Sperm Quality after Only One Spermatogenetic Cycle in Zebrafish Model. *Nutrients* 2019, 11, 843. [CrossRef]

40. Wang, G.; Chen, Q.; Tian, P.; Wang, L.; Li, X.; Lee, Y.K.; Zhao, J.; Zhang, H.; Chen, W. Gut microbiota dysbiosis might be responsible to different toxicity caused by Di-(2-ethylhexyl)phthalate exposure in murine rodents. *Environ. Pollut.* 2020, 261, 114164. [CrossRef]
41. Liu, J.-B.; Chen, K.; Li, Z.-F.; Wang, Z.-Y.; Wang, L. Glyphosate-induced gut microbiota dysbiosis facilitates male reproductive toxicity in rats. *Sci. Total Environ.* **2022**, *805*, 150368. [CrossRef]
42. Zhang, T.; Zhou, X.; Zhang, X.; Ren, X.; Wu, J.; Wang, Z.; Wang, S.; Wang, Z. Gut microbiota may contribute to the postnatal male reproductive abnormalities induced by prenatal dibutyl phthalate exposure. *Chemosphere* **2022**, *287*, 132046. [CrossRef] [PubMed]
43. Li, P.; Li, R.; Tian, X.; Zhao, Y.; Li, M.; Wang, M.; Ying, X.; Yuan, J.; Xie, J.; Yan, X.; et al. Co-exposure to fluoride and arsenic disrupts intestinal flora balance and induces testicular autoimmunity in offspring rats. *Ecotoxicol. Environ. Saf.* **2021**, *222*, 112506. [CrossRef] [PubMed]
44. Li, P.; Wei, K.; He, X.; Zhang, L.; Liu, Z.; Wei, J.; Chen, X.; Wei, H.; Chen, T. Vaginal Probiotic *Lactobacillus crispatus* Seems to Inhibit Sperm Activity and Subsequently Reduces Pregnancies in Rat. *Front. Cell Dev. Biol.* **2021**, *9*, 2116. [CrossRef]
45. Ding, N.; Zhang, X.; Di Zhang, X.; Jing, J.; Liu, S.S.; Mu, Y.P.; Peng, L.L.; Yan, Y.J.; Xiao, G.M.; Bi, X.; et al. Impairment of spermatogenesis and sperm motility by the high-fat diet-induced dysbiosis of gut microbes. *Gut* **2020**, *69*, 1608–1619. [CrossRef] [PubMed]
46. Javurek, A.B.; Spollen, W.G.; Johnson, S.A.; Bivens, N.J.; Bromert, K.H.; Givan, S.A.; Rosenfeld, C.S. Consumption of a high-fat diet alters the seminal fluid and gut microbiomes in male mice. *Reprod. Fertil. Dev.* **2017**, *29*, 1602–1612. [CrossRef] [PubMed]
47. Liu, X.; Hu, G.; Wang, A.; Long, G.; Yang, Y.; Wang, D.; Zhong, N.; Jia, J. Black Tea Reduces Diet-Induced Obesity in Mice via Modulation of Gut Microbiota and Gene Expression in Host Tissues. *Nutrients* **2021**, *14*, 1635. [CrossRef]
48. Akram, M.; Ali, S.A.; Behare, P.; Kaul, G. Dietary intake of probiotic fermented milk benefits the gut and reproductive health in mice fed with an obesogenic diet. *Food Funct.* **2021**, *13*, 737–752. [CrossRef]
49. Liu, L.; Shu, A.; Zhu, Y.; Chen, Y. Cornsilde Alleviates Diabetes Mellitus-Induced Testicular Damage by Modulating the Gut Microbiota. *Evidence-Based Complement. Altern. Med.* **2021**, *2021*, 5301942. [CrossRef]
50. Zhu, Y.; Du, Q.; Jiao, N.; Shu, A.; Gao, Y.; Chen, J.; Lv, G.; Lu, J.; Chen, Y.; Xu, H. Catalpol ameliorates diabetes-induced testicular injury and modulates gut microbiota. *Life Sci.* **2020**, *267*, 118881. [CrossRef]
51. Hao, Y.; Feng, Y.; Yan, X.; Chen, L.; Zhong, R.; Tang, X.; Shen, W.; Sun, Q.; Sun, Z.; Ren, Y.; et al. Gut microbiota-testis axis: FMT improves systemic and testicular micro-environment to increase semen quality in type 1 diabetes. *Med. Mol. 2022*, *28*, 45. [CrossRef]
52. Tian, X.; Yu, Z.; Feng, P.; Ye, Z.; Li, R.; Liu, J.; Hu, J.; Kakade, A.; Liu, P.; Li, X. *Lactobacillus plantarum* TW1-1 Alleviates Diethylhexylphthalate-Induced Testicular Damage in Mice by Modulating Gut Microbiota and Decreasing Inflammation. *Front. Cell. Infect. Microbiol.* **2019**, *9*, 221. [CrossRef] [PubMed]
53. Zhao, Y.; Zhang, P.; Ge, W.; Feng, Y.; Li, L.; Sun, Z.; Zhang, H.; Shen, W.; Alginog oligosaccharides improve germ cell development and testicular microenvironment to rescue busulfan disrupted spermatogenesis. *Theranostics* **2020**, *10*, 3306–3324. [CrossRef] [PubMed]
54. Ramirez-Acosta, S.; Selma-Royo, M.; Collado, M.C.; Navarro-Roldán, F.; Abril, N.; García-Barrera, T. Selenium supplementation influences mice testicular selenoproteins driven by gut microbiota. *Sci. Rep.* **2022**, *12*, 4218. [CrossRef] [PubMed]
55. Hou, X.; Zhu, L.; Zhang, S.; Zhang, L.; Bao, H.; Tang, M.; Wei, R.; Wang, R. Testosterone disruptor effect and gut microbiome perturbation in mice: Early life exposure to doxycycline. *Chemosphere* **2019**, *222*, 722–731. [CrossRef] [PubMed]
56. Barbonetti, A.; Cinque, B.; Vassallo, M.R.C.; Mineo, S.; Francavilla, S.; Cifone, M.G.; Francavilla, F. Effect of vaginal probiotic lactobacilli on in vitro–induced sperm lipid peroxidation and its impact on sperm motility and viability. *Fertil. Steril.* **2011**, *95*, 2488–2485. [CrossRef] [PubMed]
57. Valcarce, D.G.; Genovés, S.; Risco, M.F.; Martorell, P.; Herrera, M.P.; Ramón, D.; Robles, V. Probiotic administration improves sperm quality in asthenozoospermic human donors. *Benef. Microbes* **2017**, *8*, 193–206. [CrossRef]
58. Maretto, C.; Cavallini, G. The association of a probiotic with a prebiotic (Flortec, Bracco) to improve the quality/quantity of spermatozoa in infertile patients with idiopathic oligoasthenoteratospermia: A pilot study. *Andrology* **2017**, *5*, 439–444. [CrossRef]
59. Helli, B.; Kavianpour, M.; Ghaedi, E.; Dadfar, M.; Haghighian, H.K. Probiotic effects on sperm parameters, oxidative stress index, inflammatory factors and sex hormones in infertile men. *Hum. Fertil. 2020*, *1–9*. [CrossRef]
60. Ibrahim, H.A.M.; Zhu, Y.; Wu, C.; Lu, C.; Ezekwe, M.O.; Liao, S.F.; Haung, K. Selenium-Enriched Probiotics Improves Murine Male Fertility Compromised by High Fat Diet. *Biol. Trace Elem. Res.* **2012**, *147*, 251–260. [CrossRef]
61. Dardmeh, F.; Alipour, H.; Gazerani, P.; Van Der Horst, G.; Brandsborg, E.; Nielsen, H.I. *Lactobacillus rhamnosus* PB01 (DSM 14870) supplementation affects markers of sperm kinetic parameters in a diet-induced obesity mice model. *PLoS ONE* **2017**, *12*, e0185964. [CrossRef]
62. Guo, Y.; Du, X.; Biai, Y.; Wang, S. Chronic unpredictable stress-induced reproductive deficits were prevented by probiotics. *Reprod. Biol. 2020*, *20*, 175–183. [CrossRef] [PubMed]
63. Keshtmand, Z.; Akbaribazm, M.; Bagheri, Y.; Olaei, R. The ameliorative effects of *Lactobacillus casei* and *Lactobacillus casei* probiotics on CC4-induced testicular toxicity based on biochemical, histological and molecular analyses in rat. *Andrology* **2021**, *53*, e13908. [CrossRef] [PubMed]
64. Valdes, A.M.; Walter, J.; Segal, E.; Spector, T.D. Role of the gut microbiota in nutrition and health. *BMJ* **2018**, *361*, k2179. [CrossRef] [PubMed]
65. Flint, H.J.; Scott, K.P.; Louis, P.; Duncan, S.H. The role of the gut microbiota in nutrition and health. *Nat. Rev. Gastroenterol. Hepatol.* **2012**, *9*, 577–589. [CrossRef]
66. Rodrigues, L.E.; Kishibe, M.M.; Keller, R.; Caetano, H.R.D.S.; Rufino, M.N.; Sanches, O.D.C.; Giometti, I.C.; Giuffrida, R.; Bremer-Neto, H. Prebiotics mannan-oligosaccharides accelerate sexual maturity in rats: A randomized preclinical study. *Vet.-World 2021*, 14, 1210–1219. [CrossRef]

67. Zhang, C.; Xiong, B.; Chen, L.; Ge, W.; Yin, S.; Feng, Y.; Sun, Z.; Sun, Q.; Zhao, Y.; Shen, W.; et al. Rescue of male fertility following faecal microbiota transplantation from alginate oligosaccharide-dosed mice. *Gut 2021*, 70, 2213–2215. [CrossRef]