Review

Testicular Immunity and Its Connection with the Microbiota. Physiological and Clinical Implications in the Light of Personalized Medicine

Luigi Santacroce 1, Ciro Imbimbo 2, Andrea Ballini 3, Felice Crocetto 2, Salvatore Scacco 4,*, Stefania Cantore 5,*, Erika Di Zazzo 6, Marica Colella 1 and Emilio Jirillo 1

1 Interdisciplinary Department of Medicine, Section of Microbiology and Virology, University of Bari “Aldo Moro”, 70124 Bari, Italy
2 Department of Neuroscience, Reproductive Sciences and Dentistry, School of Medicine, University of Naples “Federico II”, 80131 Naples, Italy
3 Department of Precision Medicine, University of Campania “Luigi Vanvitelli”, 80138 Naples, Italy
4 Department of Basic Medical Sciences, Neurosciences and Sense Organs, University of Bari “Aldo Moro”, 70124 Bari, Italy
5 Independent Researcher, Sorriso & Benessere—Ricerca e Clinica, 70129 Bari, Italy
6 Department of Medicine and Health Sciences “V. Tiberio”, University of Molise, 86100 Campobasso, Italy
* Correspondence: salvatore.scacco@uniba.it (S.S.); stefaniacantore@pec.omceo.bari.it (S.C.)

Abstract: Reproduction is a complex process, which is based on the cooperation between the endocrine–immune system and the microbiota. Testicular immunity is characterized by the so-called immune privilege, a mechanism that avoids autoimmune attacks against proteins expressed by spermatozoa. Testicular microbiota is connected with the gut microbiota, the most prevalent site of commensals in the body. Both microbiotas take part in the development of the immune system and protection against pathogen invasion. Dysbiosis is caused by concurrent pathologies, such as obesity, diabetes, infections and trauma. The substitution of beneficial bacteria with pathogens may lead to destruction of spermatozoa directly or indirectly and, ultimately, to male infertility. Novel therapeutic interventions, i.e., nutritional interventions and supplementation of natural products, such as, probiotics, prebiotics, antioxidants and polyphenols, may lead to the restoration of the otherwise-impaired male reproductive potential, even if experimental and clinical results are not always concordant. In this review, the structure and immune function of the testis will be described with special reference to the blood–testis barrier. The regulatory role of both the gut and testicular microbiota will be illustrated in health and disease, also emphasizing therapeutic attempts with natural products for the correction of male infertility, in the era of personalized medicine.

Keywords: immune privilege; microbiota; male infertility; spermatogenesis; testis; polyphenols; probiotics; personalized medicine

1. Introduction

The testis is a continuous source of germ cells as the first step of male reproduction, followed by the transport of sperm to the fallopian tube sperm–egg binding sites [1]. In general terms, reproduction is a complex process, which requires a strict collaboration between the endocrine and the immune system. In fact, spermatogenesis is regulated by the hypothalamic–pituitary–testicular axis for the gonadal steroid hormone to occur [2,3]. On the other hand, the testis is endowed with a specialized immune system that becomes tolerant towards the antigenic proteins expressed by spermatozoa [4]. Such a characteristic of the testis is known as “immune privilege” in the sense that spermatozoa are protected from autoimmune attacks by gonadal immune cells [5,6]. Furthermore, the testis owns a physical barrier, the so-called blood–testis barrier (BTB), which protects germ cells from noxious immune responses [7]. Alterations of the above-described protective
homeostasis by metabolic disorders, infectious events, inflammation and trauma may lead to autoimmunity and infertility [8–10].

Microbiota is the assembly of commensal microorganisms located in different districts of the body, which contribute to the health of the host [11]. Particularly, gut microbiota, the most abundant in the body (80%), neutralizes pathogen colonization, exerts metabolic and nutritional activities and takes part in the development of the immune system [12–15]. Of note, only 9% of the human microbiota is harbored in the urogenital tract, but it is mostly gut microbiota that takes part in male and female sexual maturation. In fact, intestinal metabolites, such as secondary bile acids as well as indole and soybean, regulate male and female sexual organs [16–18].

The gut microbiota is composed of four major phyla, i.e., Bacteroidota, Bacillota, Actinomycetota and Pseudomonadota with Bacteroidota and Bacillota representing the 90% of intestinal bacteria contingent. The imbalance of gut microbiota, also in relation to high-fat and calorie-rich diets, may lead to a condition of dysbiosis, which with the time can result in disease status [19]. Moving to the male microbiota, Lactobacillus, Pseudomonas and Prevotella represent the main bacteria contained in the seminal fluid and their replacement by other pathogens may cause dysbiosis, which, in turn, leads to infertility [20].

In this review, special emphasis will be placed on the description of the immune system of the testis, as well as to the components of BTB. Then, the influence of the gut microbiota and male microbiota on the testicular immune system will be illustrated, pointing out all those conditions of dysbiosis which may alter gonadal function and fertility. Novel therapeutic attempts with natural products will also be discussed. All needs must be considered when determining the optimal way to treat an individual patient in the emerging era of personalized medicine.

1.1. The Immune Environment of the Testis

Immune privilege is confined to a few districts of the body, and, among them, the testicular environment is included. In fact, the proteins expressed on the spermatozoa membrane can elicit a robust immune attack, which may destroy spermatozoa, ultimately leading to infertility. Immune privilege relies on two major mechanisms: (I) the physical shield represented by the BTB, mainly consisting of Sertoli cells (SCs); (II) the tolerogenic response mounted by the immune armamentarium of the testis [21]. In this framework, it is important to briefly describe the structure of the testis for a better comprehension of its function. The epithelium of the seminiferous tubules holds SCs, which provide nutrition and growth factors to germ cells [22,23]. On the other hand, Leydig cells are interspersed between the tubules and secrete testosterone (T) for spermatogenesis to take place [24,25]. The interstitial space of the testis harbors lymphatic vessels, which permit access to afferent lymph nodes [26]. Peritubular lymphatic sinusoids surround the seminiferous tubules with lymphatic capillaries ubiccated beneath the tunica albuginea [27]. Macrophages, dendritic cells (DCs), mast cells and T cells are contained in the interstitium, and their function will be described in a specific section of this review. The structure of the testis is depicted in Figure 1.

1.2. Blood–Testis Barrier Function

The BTB is constituted by tight junctions (TJs), gap junctions (GJs), desmosome-like junctions and SCs. BTB is maintained by the N-cadherin/beta-catenin of the GJs and occluding/Z0-1 of the TJs all anchored in F-actin bundles [28]. Junctions prevent haploid germ cells from reaching the blood [29]. SCs supply support to germ cells, giving glucose, fatty acids and growth factors, as well as maintaining an appropriate ionic and metabolic milieu in the testis [30,31]. Moreover, SCs secrete factors which keep an immunoprotected environment in the testis, such as transforming growth factor (TGF)-beta 1 in order to avoid autoimmune destruction of sperm cells [32].

Another mechanism of protection elicited by SCs is their ability to phagocyte apoptotic germ cells and residual bodies [33]. In order to accomplish their phagocytic activity, SCs
utilize Axl, tyro3 and Mer tyrosine kinase receptors, as well as growth-arrest-specific gene 6 (GAS6). SC-mediated phagocytosis supports spermatogenesis through various mechanisms: (1) making room for the germ cell differentiation process; (2) eliminating harmful substances derived from necrotic germ cells; (3) clearing autoantigens, which may trigger autoimmune responses; (4) providing energy sources to other SCs via the recycle of apoptotic germ cell components [34]. Lastly, SCs switch off the inflammatory responses of T cells in the testicular interstitium [35]. The BTB is illustrated in Figure 2.

1.3. The Immune Arsenal of the Testis

The BTB via SCs constitutes a physical barrier devoted to the protection of germ cells from a destructive immune attack. Besides that, testicular immune cells maintain either a tolerogenic milieu or protect spermatogenesis from pathogen invasion; thus, they control inflammatory processes, which very often are responsible for male infertility [36–38]. In fact, evidence has

![Figure 1. Structure of the testis. The structure of the testis consists of the epithelium of the seminiferous tubules and the interstitium. In turn, the interstitium harbors Leydig cells, immune cells and lymphatic vessels.](image)

![Figure 2. Blood–testis barrier composition. The blood–testis barrier, besides in gap junctions, desmosome-like junctions and tight junctions, has Sertoli cells (SCs). SCs participate in the nutrition and growth of germ cells, maintenance of the immune privilege and clearance of autoantigens and apoptotic cells via phagocytosis.](image)
been provided that infections or inflammatory states inhibit steroidogenesis, cause apoptosis of germ cells and destroy testicular epithelial cells, thus provoking infertility [39].

Immune response relies on two major arms, the innate immune system and the adaptive immune system, respectively [40]. Phagocytes (granulocytes and macrophages), natural killer cells and dendritic cells (DCs) [major antigen-presenting cells (APCs)] are involved in the innate immune response. On the other hand, T and B lymphocytes recognize their specific antigens and maintain immunological memory. Mostly, T cells are divided into different subsets, such as T helper (h), T cytotoxic (Tc) and T regulatory (Treg) cells [41].

In the next paragraphs, the functions of testicular macrophages, DCs and lymphocytes will be discussed under both steady state and inflammatory conditions.

(a) Macrophages

Testicular macrophages derive from three distinct sources: (1) early yolk sac macrophages; (2) fetal liver monocytes; (3) bone-marrow-derived monocytes [42,43]. Experimental studies have reported that testicular macrophages are able to preserve the local immune privilege, as observed in the testis of rats where these activated phagocytes produce the anti-inflammatory cytokine, interleukin (IL)-10, also expanding T regulatory (Treg) cells [44,45].

Testicular inflammation is caused either by bacteria such as Escherichia (E.) coli and Klebsiella spp. or viruses (HIV-1, Zika and Mumps orthorubulavirus) [46,47]. Furthermore, in this instance, animal experiments have clarified the detrimental role of infiltrating monocyte-derived macrophages in the promotion of local inflammation, even if the differentiation of peripheral monocytes into testicular macrophages needs further demonstration [48]. Moreover, infected testicular macrophages have been shown to alter SC TJ and interrupt the BTB [49].

Testicular macrophages have been divided into three groups: (1) ED-1 recognizing macrophages, a class of pro-inflammatory cells, which produce tumor necrosis factor-alpha and interferon-gamma; (2) ED-2 macrophages, which exert anti-inflammatory activities by release of IL-10; (3) ED1+ED-2 macrophages, which are a source of nitric oxidase synthase (NOS) [46,47]. ED2+ cells are the majority of the testis macrophages that support a tolerogenic milieu in this organ [50].

(b) Dendritic Cells

Dendritic cells (DCs), as professional antigen-presenting cells (APCs), play a tolerogenic effect in the testis, principally leading to Treg cell activation in response to normal sperm antigens [51]. Furthermore, indoleamine 2,3-dioxygenase (IDO), which catalyzes the tryptophan metabolism and generates kynurenine, has been found in activated DCs, thus contributing to immune privilege [52]. In fact, kynurenine, acting as a ligand for aryl hydrocarbon receptors on T cells, induces the generation of Foxp3+ Treg cells [51]. Of note, IDO has been shown to induce Treg cell activation in tumors and pregnant uterus, which are also privileged sites, like the testis [53,54].

Under pathological circumstances, in azoospermic humans testicular DCs are able to activate autoreactive T cells, upregulating co-stimulatory molecules, proinflammatory cytokines and major histocompatibility complex class-II (MHC-II), thus leading to male infertility [55,56].

(c) T Cells

Treg cells (T cells) have been detected in the mouse, rat and human testis, where they reside in the draining lymph nodes, thus interacting with tissue-specific autoantigens [57]. Located in such a strategic position, Treg cells exert their suppressive function, thus protecting spermatozoa from autoimmune attacks. In this respect, patients with autoimmune regulator gene mutation associated to a defect of Treg cells undergo a chronic testicular inflammation [58]. In chronic inflamed human azoospermic testis, evidence has been provided that Foxp3+ Treg cells are decreased with an increase in the proinflammatory T cell subset, T helper (h) 17 cells [59]. In rat experimental autoimmune orchitis (EAO), CD8+, CD25+, Foxp3+ and CD4+, CD25+, Foxp3+ and Treg cells are increased in the early phase, while the latter subset decreases in the chronic phase [60].
All the above evidence suggests that Treg cells are overly critical in the prevention of organ-specific autoimmunity and maintenance of the immune privilege in the testis. Testicular Th1 cells seem to be necessary for supporting immune homeostasis in this organ. However, an excessive activation of these cells may contribute to autoimmune orchitis [61]. Further studies have proven the intervention of Th17 cells in the later phase of autoimmune orchitis, thus hampering the function of Treg cells, also contributing to the subversion of the testis structure and spermatogenesis [62].

T cytotoxic (c) lymphocytes (CD8+ cells) harbor the testis in a percentage which is 2-fold higher than that of Th cells [63]. Testicular CD8+ cells are functionally associated with resident macrophages or Leydig cells and take part in graft survival [64]. In this respect, pancreatic and islet transplantation in the testis undergoes a lower rate of rejection with an elevated induction of Treg cells [65, 66]. This may depend on the SC-mediated activation of Treg cells or on the less potent cytotoxic activity of testicular Tc lymphocytes [67]. To complete the above issue, it is worth mentioning the relationship between T lymphocytes, Leydig cells and SCs, respectively. Leydig cells harbor the interstitial region between seminiferous tubules and represent the major source of T [68]. Co-cultures of Leydig cells and T cellshave revealed the suppressive effect of the former on the latter, also in view of the binding of Leydig cells to T cells via vascular adhesion molecules [69, 70]. Androgen receptors are expressed on T cells and, therefore, Leydig cells can modulate their function through androgen secretion.

Experimentally, depletion of T by ethane dimethane sulphonate gives rise to an epididymal sperm granuloma and accumulation of CD4+ and CD8+ T cells, which can be abrogated by supplementation of T [71]. Furthermore, in the EAO rat model, T replacement inhibits the development of autoimmune orchitis through the expansion of Treg cells [72]. Conclusively, Leydig cells are able to limit the infiltration of T cells within the testis, directly and indirectly. SCs are devoted to the protection of spermatogenesis, acting as immunological sentinels. In this regard, it appears that SCs promote the differentiation of tolerogenic DCs and Treg cells [73]. Of note, SCs behave as nonprofessional APCs, expressing MHC-II molecules, thus mediating the expansion of Foxp3+ Treg cells [74].

In this direction, transplanted SCs protect syngeneic islet grafts, generating Treg cells and decreasing release of IL-17 by T helper (h)17 cells [75]. In sum, SCs not only contribute to the BTB composition but also keep on check detrimental T cell responses. The testicular immune cells are expressed in Figure 3.

![Figure 3: Macrophages, Dendritic cells, and T cells sustain the testicular immune response.](image)

Figure 3. Macrophages, Dendritic cells, and T cells sustain the testicular immune response. ED-2 macrophages, DCs and Treg cells keep immune privilege. Conversely, ED-1 macrophages and Th17 cells take part in chronic orchitis progression. Leydig cells attenuate autoimmune orchitis via release of testosterone. SCs maintain the immune privilege via activation of tolerogenic DCs and Treg cells.
1.4. Composition and Function of the Testicular Microbiota

The dogma according to which the testis is an immune privileged site has been contradicted by the evidence that a few bacteria are able to colonize the gonad milieu. In fact, the phyla *Actinomycetota*, *Bacteroidota*, *Bacillota* and *Pseudomonadota* have been detected in testicular biopsies of azoospermic patients [76]. Moreover, the phyla *Bacillota*, *Actinomycetota*, *Bacteroidota* and the genera *Blautia*, *Clostridium* and *Prevotella* were found in testicular specimens of infertile men [77]. In another report, in dyspermic patients and healthy donors *Lactobacillus*, *Pseudomonas*, *Prevotella* and the phyla *Pseudomonadota*, *Bacillota*, *Actinomycetota*, *Bacteroidota* and *Fusobacteria* were identified, with the genus *Prevotella* being inversely associated with sperm concentration, while the *Pseudomonas* genus was correlated with sperm motility [78,79].

Despite the detection of the testicular microbiota, its role in the testis is still debated. According to a recent report, testicular microbiota seems to expand IL-17, producing gamma-delta T cells during puberty, promoting gonadal immune surveillance [80]. It is noteworthy that current research in this specific field has been focused on the link between gut microbiota and testicular microbiota. In the zebrafish model, the genera *Vibrio*, *Aeromonas*, *Pseudomonas* and *Plesiomonas* spp. have been detected in both gut and testis [81,82]. In the same model, excessive fat intake led to a dramatic reduction of the genus *Vibrio* and *Plesiomonas* spp., with a subversion of signal transduction mechanisms, amino acid transport and metabolism. Furthermore, testicular microbiota regulates the signaling mechanisms of vitamin K and vitamin A and its alteration may change the composition of the extracellular matrix, ultimately leading to male infertility [83,84].

In this direction, evidence has been provided that in a metabolic syndrome model, vitamin A deficit alters the gut-testis axis, finally resulting in an impaired spermatogenesis [85,86]. The gut-testis axis is supported by experimental evidence. Transplantation of fecal flora from high-fat diet (HFD) to normal mice caused an increase in *Bacteroidota* phylum and *Prevotella* genus in normal mice followed by intestinal inflammation and endotoxemia, but mostly by an impaired spermatogenesis [82,87]. In the human counterpart, male infertility is characterized by a negative correlation between *Bacteroidota* phylum and *Prevotella* genus with sperm viability as a result of the “leaky gut hypothesis”. Thus, intestinal endotoxins may impede the T synthesis in Leydig cells, thus provoking a decrease in spermatozoa [88]. More precisely, endotoxins via binding to the TLR-4 expressed on immune cells and epithelial cells can activate the NF-kB pathway with massive release of proinflammatory cytokines [89,90]. In turn, cytokines activate the xanthine oxidase system, thus generating, elevated levels of reactive oxygen species (ROS) and oxidative stress [91].

Conclusively, the bacterial translocation-mediated inflammation can account for endothelial damage, subversion of the BTB and alteration of the spermatogenesis and spermatozoa viability [92]. Additionally, DCs and macrophages, which infiltrate the epididymis, are able to capture spermatozoa, thus, contributing, to the impairment of spermatogenesis [93]. Another link between gut microbiota and male reproduction is represented by the endotoxin-mediated insulin resistance (IR), as an expression of altered intestinal permeability [94–96]. IR stands for an event of pathogenetic relevance since it alters both gut microbiota and spermatogenesis. In fact, in infertility models with IR, higher levels of *Saccharibacteria* phylum and lower levels of the phyla *Actinomycetota* and *Verrucomicrobia* have been observed in comparison to controls without IR [97]. Parallelly, increased IR is associated with a decreased secretion of T by Leydig cells also in view of a reduced gonadotropin release [98]. In Figure 4 the gut-testis axis is described.
Acute epididymitis is a very frequent infection of the male reproductive tract, even if this genus was decreased [100].

Figure 4. Fecal transplantation from high-fat diet mice to normal mice accounts for endotoxemia occurrence and altered spermatogenesis. In turn, endotoxemia abrogates synthesis of testosterone from Leydig cells, thus reducing the number of spermatozoa with increased release of pro-inflammatory cytokines. Inflammatory seminal fluid is associated with male microbiota.

1.5. Seminal Dysbiosis with Particular Reference to Male Infertility

The influence of seminal dysbiosis is an issue of current interest. Dyspermic conditions, i.e., oligo-azoospermia, asteno-azoospermia and azoospermia, have been investigated in terms of microbial composition of seminal fluid. For instance, in azoospermic individuals, Bacteroidota and Bacillota phyla are increased, while the phyla Pseudomonadota and Actinomycetota are reduced [98]. In the oligo-asteno-teratozoospermic patients, instead, the genera Neisseria, Klebsiella, and Pseudomonas and the phylum Bacillota are very abundant, but there is a decrease in Lactobacillus [99]. In idiopathic non-obstructive azoospermic patients, the Clostridium genus was decreased [100].

Quite interestingly, over the past few years, the influence of female microbiota on the male microbiota has intensively been investigated. For instance, Gardnerella vaginalis and the genus Lactobacillus have been detected in younger men’s seminal microbiota, while the genera Pseudomonas, Flavobacterium and Acidovorax have been found in seminal fluid of older individuals [101, 102]. On the other hand, inflammatory seminal fluid is associated with Streptococcus agalactiae, Gardnerella vaginalis and bacterial vaginosis-related bacteria [103].

1.6. Microbial-Mediated Male Infertility

Despite the presence of the BTB, the testicular immune arsenal and the local microbiota, the testis can be invaded by urethral pathogens and sexually transmitted bacteria [104]. Acute epididymitis is a very frequent infection of the male reproductive tract, even if this organ has a structure quite overlapping that of the testis [105]. More exactly, epididymis is a less immunologically privileged site in comparison to the testis with a certain degree of immune responsiveness in the caput and an inflammatory profile in the cauda [106, 107]. In epididymitis patients, the quality of semen is very low, with an alteration of the protein com-
position of the sperm, thus contributing to male infertility [108,109]. Persistent pathogen damage leads to fibrotic transformation and epithelial degeneration of the epididymis [110].

Due to the scarcity of human epididymal specimens, research has mainly been conducted on rodent tissue samples. Experimental Gram-negative and Gram-positive infections in the mouse testis have revealed a strong proinflammatory cytokine response with upregulation of NOS-2 [111]. Uropathogenic Escherichia coli (UPEC) infections in the mouse are characterized by an activation of TLR4 and TLR5 in the epididymis caput with liberation of proinflammatory cytokines and type 1 interferon [112]. On the other hand, epididymal cells respond to UPEC challenge with the production of the antimicrobial peptide defensin b2, which is more effective than gentamycin in reducing bacterial load in both epididymis and testis [113].

*Chlamydia trachomatis (Ct)* is the most frequent sexually transmitted pathogen in males, leading to chronic inflammation and scarring of the male genital tract [114]. Ct antigens bind to TLR2 and TLR4 and pathogen recognition receptors with massive liberation of proinflammatory cytokines, which account for chronicity of inflammation [115]. As far as viral diseases are concerned, mumps virus, an RNA virus, is the most frequent cause of epididymitis and orchitis, which in turn cause male infertility [116]. COVID-19 has been reported to infect the testis, impairing T secretion, thus inducing primary hypogonadism or aggravating a preexistent status of hypogonadism [117]. In particular, a reduced number of Leydig cells have been detected in COVID-19 patients along with a high expression of angiotensin-converting enzyme 2 in the testis [118–121]. Additionally, involvement of testicular T and B lymphocytes in COVID-19 infection has been reported [122]. Infections of the male reproductive tract are illustrated in Figure 5.

**UROPATHOGENIC E.COLI (UPEC) INFECTION OF THE EPIDIDYMIS**

- Activation of TLR4 and TLR5 → Pro-inflammatory cytokines
- Nitric oxide synthase → Nitric oxide
- Fibrotic transformation and epithelial degeneration

**Chlamydia trachomatis**

Activation of TLR2 and TLR4 → pro-inflammatory cytokine -> scarring of the male genital tract

**COVID-19 infection**

- Reduced number of Leydig cells and high expression of angiotensin-converting enzyme 2 in the testis
- Infiltration of T and B lymphocytes in testis

*Figure 5.* Microbial invasion of the testis. UPEC and *C. trachomatis* damage the male genital tract via production of pro-inflammatory cytokines and nitric oxide, with tissue scarring. COVID-19 infection is characterized by a reduction of Leydig cells, infiltration of T and B cells and elevated expression of ACE-2.

1.7. Therapeutic Correction of Testicular Dysbiosis with Natural Products

In view of the connection between microbiota and male reproduction new therapies have been explored. With special reference to personalized medicine, there is a large body of evidence that nutrition can influence the composition of the microbiota, the quality of sperm in terms of caloric content of food components, as well as fatty acid, carbohydrate and protein profiles. In this regard, a high intake of saturated fatty acids may impair male fertility, while a healthy dietary regimen, i.e., the Mediterranean diet (MED) contributes to the preservation of the microbiota and sperm quality [123–125]. Conversely,
the Western diet causes the rapid spread of obesity associated with hyperinsulinemia and hyperglycemia, which lead to an altered sperm function [126,127].

On these grounds, nutritional interventions to protect male reproduction have been adopted. For instance, MED has been shown to positively affect male reproductive performance, especially through the consumption of extra virgin olive oil (EVOO). According to [128], EVOO is able to change the sperm membrane lipid composition, reducing oxidative stress and enhancing mitochondrial function. Furthermore, MED exerts a homeostatic function in the endocrine–metabolic–immune axis, also shifting the gut microbiota towards an anti-inflammatory profile [129,130]. It is likely that testicular microbiota may be positively affected by MED, but such an assumption needs scientific demonstration. Certain natural products potentially effective in the restoration of testicular microbiota will be illustrated in the following paragraphs.

(a) Probiotics

Probiotics by definition are “live microorganisms which when administered in adequate amounts confer a health benefit on the host” [131,132]. They have been used to enhance male reproduction, owing to their ability to protect the intestinal barrier, inhibit pathogen growth and activate the immune response [133,134]. In astheno-azoospermic human donors, 3 weeks’ supplementation of *Lactobacillus* (*L.*) *rhamnosus* and *Bifidobacterium longum* improved sperm motility while reducing DNA fragmentation [135,136]. In another study, administration of a symbiotic, Familac®, composed by *Lactobacillus* strains and oligo-fructosaccharides, to idiopathic male infertility could enhance sperm quality and DNA integrity, while reducing free radicals in the semen [137].

Experimentally, in HFD obese mice, supplementation of *L. rhamnosus* improved spermatozoa motility, increasing the number of Leydig cells [138]. In infertile mice, administration of *Lactobacillus* spp., *Bacillus* spp., *Saccharomyces cerevisiae* (beer yeast) and photosynthetic bacteria cultures reduced sperm damage and improved motility [139].

(b) Prebiotics

Oligofructose, galacto-oligosaccharides and breast-milk oligosaccharides are the most representative prebiotics, endowed with the ability to increase levels of *Bifidobacterium* and *Lactobacillus*, as well as of SCFAs [140,141]. In a preclinical study, evidence has been provided that manno-oligosaccharides were able to accelerate sexual maturation in rats [142]. In particular, the decrease in blood corticosterone observed in this study could account for the elevated levels of T and maturation of seminiferous tubules. To the best of our knowledge, no clinical trials have been conducted to treat male infertility with prebiotics.

(c) Antioxidants

Vitamin C and vitamin E can exert especially beneficial effects in infertile men, reducing ROS levels, improving sperm motility and maintaining DNA integrity [143]. Among other antioxidants, lycopene, present in tomatoes and red fruits, seems to display positive effects on the testicular mitochondria by modulating lipid peroxidation within the mitochondrial membrane [144]. Conversely, other studies based on the administration of antioxidants did not show any improvement of semen biomarkers and DNA integrity in infertile men [145,146].

(d) Polyphenols

Polyphenols are natural compounds, mainly contained in fruits, vegetables, oil, wine and cocoa [147,148]. They exert potent anti-inflammatory and antioxidant activities on different cell types, even including spermcells [149–151]. Experimental and human studies have been undertaken with quercetin and resveratrol; however, results have been quite controversial, since both polyphenols are endowed with antioxidant and pro-oxidant activities [152,153].

Table 1 shows the main natural products putatively involved in the treatment of male genital tract infections.
Table 1. Natural products for the correction of testicular dysbiosis. Both probiotics and synbiotics are able to improve sperm quality and motility in male infertility. In rats, manno-oligosaccharides (prebiotics) promote sexual maturity. Polyphenols exert both antioxidant and pro-oxidant activities. Among antioxidants, vitamin C and vitamin E are able to reduce ROS generation and improve sperm mobility and DNA integrity. Lycopene enhances sperm performance via lipid peroxidation on mitochondrial membranes.

| PROBIOTICS                          | Natural Products against Testicular Dysbiosis |
|-------------------------------------|------------------------------------------------|
| • In astenozoospermic human donors   | Prebiotics                                      |
| • In idiopathic male infertility    | Polyphenols                                    |
| • Lactobacillus-mediated            | Anti-oxidants                                  |
| improvement of enhancement          | • Vitamin C and vitamin E ->                   |
| of sperm quality and reduction of   | • reduction of ROS and improvement             |
| sperm motility and DNA              | • of sperm mobility and DNA integrity          |
| fragmentation free radicals in the  | • Lycopene -> modulaion of lipid               |
| semen                               | peroxidation on mitochondrial membrane         |

2. Conclusions

A mutual cooperation between testicular immunity and microbiota contribute to normal spermatogenesis and sperm maturation. Such an equilibrium may be subverted by a range of factors, even including concurrent pathologies, e.g., obesity, diabetes, infections and trauma. Among novel therapeutic approaches to restore male infertility, a proper nutritional regimen, as in the case of MED, may be useful in male infertility associated to obesity and diabetes. Furthermore, supplementation of natural products, such as probiotics, prebiotics, antioxidants and polyphenols has been demonstrated to enhance male reproductive function either in animal models or clinical trials. However, also, in view of conflicting results, more clinical attempts are needed to establish the actual effectiveness of natural products for the correction of testicular microbiota and immune function in the era of personalized medicine.

Author Contributions: Conceptualization, E.J., A.B. and L.S.; supervision and project administration, C.I., F.C., E.D.Z., M.C. and S.C.; revising the work critically for important intellectual content, E.J., A.B., S.C. and L.S.; final approval of the version to be published, E.J., C.I., A.B. and L.S.; validation and bibliographic research, S.C. and S.S.; acquisition and interpretation of data for the work, S.C. and E.D.Z.; agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved, all authors. 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.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors thank Ioannis A. Charitos for his valuable help in preparing the figures of the text.

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

References

1. Agarwal, A.; Barbăroșie, C.; Ambar, R.; Finelli, R. The Impact of Single- and Double-Strand DNA Breaks in Human Spermatozoa on Assisted Reproduction. *Int. J. Mol. Sci.* 2020, 21, 3882. [CrossRef] [PubMed]
2. Hermann, B.P.; Cheng, K.; Singh, A.; Roa-De La Cruz, L.; Mutoji, K.N.; Chen, I.C.; Gildersleeve, H.; Lehle, J.D.; Mayo, M.; Westernstroer, B.; et al. The Mammalian Spermatogenesis Single-Cell Transcriptome, from Spermatogonial Stem Cells to Spermatids. *Cell Rep.* 2018, 25, 1650–1667.e8. [CrossRef] [PubMed]
3. Ye, L.; Zeng, Q.; Ling, M.; Ma, R.; Chen, H.; Lin, F.; Li, Z.; Pan, L. Inhibition of IP3R/Ca\(^{2+}\) Dysregulation Protects Mice from Ventilator-Induced Lung Injury via Endoplasmic Reticulum and Mitochondrial Pathways. *Front. Immunol.* 2021, 12, 729094. [CrossRef] [PubMed]
4. Meinhardt, A.; Wang, M.; Schulz, C.; Bhushan, S. Microenvironmental signals govern the cellular identity of testicular macrophages. *J. Leukoc. Biol.* 2018, 104, 757–766. [CrossRef]

5. Forresters, J.V.; Xu, H.; Lambe, T.; Cornall, R. Immune privilege or privileged immunity? *Mucosal Immunol.* 2008, 1, 372–381. [CrossRef]

6. O’Donnell, L.; Smith, L.B.; Reboulec, D. Sperm-specific proteins: New implications for diagnostic development and cancer immunotherapy. *Curr. Opin. Cell Biol.* 2022, 77, 10214. [CrossRef]

7. Stanton, P.G. Regulation of the blood-testis barrier. *Semin. Cell Dev. Biol.* 2016, 59, 166–173. [CrossRef]

8. Schuppe, H.C.; Meinhardt, A. Immune privilege and inflammation of the testis. *Immunol. Gametes Embryo Implant.* 2005, 88, 1–14. [CrossRef]

9. Chen, Q.; Deng, T.; Han, D. Testicular immunoregulation and spermatogenesis. *Semin. Cell Dev. Biol.* 2016, 59, 157–165. [CrossRef]

10. Loveland, K.L.; Klein, B.; Pueschl, D.; Indumathy, S.; Bertram, M.; Loveland, B.E.; Hedger, M.P.; Schuppe, H.C. Cytokines in Male Fertility and Reproductive Pathologies: Immunoregulation and Beyond. *Front. Endocrinol.* 2017, 8, 307. [CrossRef]

11. Proctor, L. Priorities for the next 10 years of human microbiome research. *Nature* 2019, 569, 623–625. [CrossRef] [PubMed]

12. O’Hara, A.M.; Shanahan, F. The gut flora as a forgotten organ. *EMBO Rep.* 2006, 7, 688–693. [CrossRef] [PubMed]

13. Belkaid, Y.; Harrison, O.J. Homeostatic Immunity and the Microbiota. *Immunity* 2017, 46, 562–576. [CrossRef] [PubMed]

14. Desmet, L.; Thijs, T.; Segers, A.; Verbeke, K.; Depoortere, I. Chronodisruption by chronic jetlag impacts metabolic and gastrointestinal homeostasis in male mice. *Acta Physiol.* 2021, 233, e13703. [CrossRef]

15. Zhang, Z.J.; Lehmann, C.J.; Cole, C.G.; Pamer, E.G. Translating Microbiome Research from and to the Clinic. *Annu. Rev. Microbiol.* 2022. [CrossRef]

16. Baptissart, M.; Vega, A.; Martinot, E.; Pommier, A.J.; Houten, S.M.; Marceau, G.; de Haze, A.; Baron, S.; Schoonjans, K.; de Vos, W.M.; et al. Indoles from commensal bacteria extend healthspan. *Proc. Natl. Acad. Sci. USA* 2017, 114, E7506–E7515. [CrossRef]

17. Sonowal, R.; Swimm, A.; Luo, L.; Matsunaga, Y.; Wu, Z.; Bhringade, J.A.; Ejzak, E.A.; Ranawade, A.; Qadota, H.; et al. Bile acids alter male fertility through G-protein-coupled bile acid receptor 1 signaling pathways in mice. *Hepatology* 2014, 60, 1054–1065. [CrossRef]

18. Sonowal, R.; Swimm, A.; Luo, L.; Matsunaga, Y.; Wu, Z.; Bhringade, J.A.; Ejzak, E.A.; Ranawade, A.; Qadota, H.; et al. Indoles from commensal bacteria extend healthspan. *Proc. Natl. Acad. Sci. USA* 2017, 114, E7506–E7515. [CrossRef]

19. Selvaraj, V.; Zakroczymski, M.A.; Naza, A.; Mukai, M.; Ju, Y.H.; Doerge, D.R.; Katzenellenbogen, J.A.; Helferich, W.G.; Cooke, P.S. Estrogenicity of the isoflavone metabolite equol on reproductive and non-reproductive organs in mice. *Biol. Reprod.* 2004, 71, 966–972. [CrossRef]

20. Nobs, S.P.; Zmora, N.; Elinav, E. Nutrition Regulates Innate Immunity in Health and Disease. *Annu. Rev. Nutr.* 2020, 40, 189–219. [CrossRef]

21. Lundy, S.D.; Vij, S.C.; Eng, C. Reply to Eugenio Ventimiglia, EdoardoPozzi, Massimo Alfiano, Francesco Montorsi, and Andrea Salonia’s Letter to the Editor re: Scott D. Lundy, Naseer Sangwan, Neel V. Parekh; et al. Functional and Taxonomic Dysbiosis of the Gut, Urine, and Semen Microbiomes in Male Infertility. *Eur Urol* 2021, 79, 826–36. *Eur. Urol.* 2021, 80, e55–e56. [CrossRef]

22. Ruthig, V.A.; Lamb, D.J. Updates in Sertoli Cell-Mediated Signaling During Spermatogenesis and Advances in Restoring Sertoli Cell Function. *Front. Endocrinol.* 2022, 13, 897196. [CrossRef] [PubMed]

23. Meinhardt, A.; Hedger, M.P. Immunological, paracrine and endocrine aspects of testicular immune privilege. *Mol. Cell. Endocrinol.* 2011, 335, 60–68. [CrossRef] [PubMed]

24. Li, N.; Wang, T.; Han, D. Structural, cellular and molecular aspects of immune privilege in the testis. *Front. Immunol.* 2012, 3, 152. [CrossRef] [PubMed]

25. Lu, N.; Wang, T.; Han, D. Structural, cellular and molecular aspects of immune privilege in the testis. *Front. Immunol.* 2012, 3, 152. [CrossRef] [PubMed]

26. Meinhardt, A.; Hedger, M.P. Immunological, paracrine and endocrine aspects of testicular immune privilege. *Mol. Cell. Endocrinol.* 2011, 335, 60–68. [CrossRef] [PubMed]

27. Fijak, M.; Bhushan, S.; Meinhardt, A. Immunoprivileged sites: The testis. *Methods Mol. Biol.* 2011, 677, 459–470. [CrossRef]

28. Hirai, S.; Naito, M.; Terayama, H.; Qu, N.; Kuerban, M.; Musha, M.; Ikeda, A.; Miura, M.; Itoh, M. The origin of lymphatic capillaries in murine testes. *J. Androl.* 2012, 33, 745–751. [CrossRef]

29. Su, L.; Wang, Z.; Xie, S.; Hu, D.; Cheng, Y.C.; Mruk, D.D.; Guan, Y. Testin regulates the blood-testis barrier via disturbing occludinZO-1 association and actin organization. *J. Cell. Physiol.* 2020, 235, 6127–6138. [CrossRef]

30. Mital, P.; Hinton, B.T.; Dufour, J.M. The blood-testis and blood-epididymis barriers are more than just their tight junctions. *Biol. Reprod.* 2011, 84, 851–858. [CrossRef]

31. Rato, L.; Alves, M.G.; Socorro, S.; Duarte, A.I.; Cavaco, J.E.; Oliveira, P.F. Metabolic regulation is important for spermatogenesis. *Nat. Rev. Urol.* 2012, 9, 330–338. [CrossRef]

32. Ye, L.; Huang, W.; Liu, S.; Cai, S.; Hong, L.; Xiao, W.; Thiele, K.; Zeng, Y.; Song, M.; Diao, L. Impacts of Immunometabolism on Male Reproduction. *Front. Immunol.* 2021, 12, 658432. [CrossRef] [PubMed]

33. Suarez-Pinzon, W.; Korbultt, G.S.; Power, R.; Hooton, J.; Rajotte, R.V.; Rabinovitch, A. Testicular sertoli cells protect islet beta-cells from autoimmune destruction in NOD mice by a transforming growth factor-beta1-dependent mechanism. *Diabetes* 2000, 49, 1810–1818. [CrossRef]

34. Nakanishi, Y.; Shiratsuchi, A. Phagocytic removal of apoptotic spermatogenic cells by Sertoli cells: Mechanisms and consequences. *Biol. Pharm. Bull.* 2004, 27, 13–16. [CrossRef] [PubMed]
34. Dutta, S.; Sengupta, P.; Slama, P.; Roychoudhury, S. Oxidative Stress, Testicular Inflammatory Pathways, and Male Reproduction. *Int. J. Mol. Sci.* 2021, 22, 10043. [CrossRef] [PubMed]
35. Zhao, S.; Zhu, W.; Xue, S.; Han, D. Testicular defense systems: Immune privilege and innate immunity. *Cell. Mol. Immunol.* 2014, 11, 429–437. [CrossRef] [PubMed]
36. Punab, M.; Poomets, O.; Paju, P.; Vihlajav, V.; Pomm, K.; Ladva, R.; Korrobits, P.; Laan, M. Causes of male infertility: A 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. *Hum. Reprod.* 2017, 32, 18–31. [CrossRef]
37. Michiel, V.; Duan, Y.; Stoschek, E.; Bhushan, S.; Middendorff, R.; Young, J.M.; Loveland, K.L.; Kretser, D.M.; Hedger, M.P.; Meinhardt, A. Uropathogenic Escherichia coli causes fibrotic remodelling of the epididymis. *J. Pathol.* 2016, 240, 15–24. [CrossRef] [PubMed]
38. Schuppe, H.C.; Pilat, A.; Hossain, H.; Diemner, T.; Wagenerlehner, F.; Weidner, W. Urogenital Infection as a Risk Factor for Male Infertility. *Dtsch. Arzteblatt Int.* 2017, 114, 339–346. [CrossRef]
39. Hedger, M.P. Immunophysiology and pathology of inflammation in the testis and epididymis. *J. Androl.* 2011, 32, 625–640. [CrossRef]
40. Gray, J.L.; Farber, D.L. Tissue-Resident Immune Cells in Mammals. *Annu. Rev. Immunol.* 2020, 40, 195–220. [CrossRef]
41. Ivanov, I.; Tuganbaev, T.; Skelly, A.N.; Honda, K. T Cell Responses to the Microbiota. *Annu. Rev. Immunol.* 2022, 40, 559–587. [CrossRef] [PubMed]
42. Ginhoux, F.; Greter, M.; Leboeuf, M.; Nandi, S.; See, P.; Gokhan, S.; Mehler, M.F.; Conway, S.J.; Ng, L.G.; Stanley, E.R.; et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 2010, 330, 841–845. [CrossRef] [PubMed]
43. Gomez Perdigueró, E.; Fléchet, K.; Schulz, C.; Busch, K.; Azzoni, E.; Crozet, L.; Garner, H.; Trouillet, C.; de Bruin, M.F.; Geissmann, F.; et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* 2015, 518, 547–551. [CrossRef]
44. Meinhardt, A.; Dejouc-Rainsford, N.; Bhushan, S. Testicular macrophages: Development and function in health and disease. *Trends Immunol.* 2022, 43, 51–62. [CrossRef] [PubMed]
45. Wang, M.; Fijak, M.; Hossain, H.; Markmann, M.; Nüssing, R.M.; Lochnit, G.; Hartmann, M.F.; Wudy, S.A.; Zhang, L.; Gu, H.; et al. Characterisation of the Micro-Environment of the Testis that Shapes the Phenotype and Function of Testicular Macrophages. *J. Immunol.* 2017, 198, 4327–4340. [CrossRef] [PubMed]
46. Pleuger, C.; Silva, E.J.R.; Pilat, A.; Bhushan, S.; Meinhardt, A. Differential Immune Response to Infection and Acute Inflammation Along the Epididymis. *Front. Immunol.* 2020, 11, 599594. [CrossRef] [PubMed]
47. Ali, B.R.; Atiyah, S.A.; Yser, H.T.; Khelewe, A.M.; Hameed, H.N. The influence of SARS-CoV-2 on semen parameters of infected fertile male in comparison with those that noninfected. *J. Clin. Lab. Anal.* 2022, e24568. [CrossRef]
48. Tsitsarkin, K.A.; Acklin, J.A.; Liu, G.; Kenney, H.; Teterina, N.L.; Pletnev, A.G.; Lim, J.K. Zika virus tropism during early infection of the testicular interstitium and its role in viral pathogenesis in the testes. *PLoS Pathog.* 2020, 16, e1008601. [CrossRef]
49. Bhushan, S.; Meinhardt, A. The macrophages in testis function. *J. Reprod. Immunol.* 2017, 119, 107–112. [CrossRef]
50. Jarazo-Dietrich, S.; Jacobo, P.; Perez, C.V.; Guazzzone, V.A.; Lustig, L.; Theas, M.S. Up regulation of nitric oxide synthase-nitric oxide system in the testis of rats undergoing autoimmune orchitis. *Immunobiology* 2012, 217, 778–787. [CrossRef]
51. De Rose, R.; Fernandez, C.S.; Hedger, M.P.; Kent, S.J.; Winnall, W.R. Characterisation of macaque testicular leucocyte populations and T-lymphocyte immunity. *J. Reprod. Immunol.* 2013, 100, 146–156. [CrossRef] [PubMed]
52. Wang, P.; Duan, Y.G. The role of dendritic cells in male reproductive tract. *Am. J. Reprod. Immunol.* 2016, 76, 186–192. [CrossRef] [PubMed]
53. Gualdoni, G.S.; Jacobo, P.V.; Sobarlo, C.M.; Perez, C.V.; Matzkin, M.E.; Höcht, C.; Frungieri, M.B.; Hill, M.; Anecon, I.; Lustig, L.; et al. Role of indoleamine 2,3-dioxygenase in testicular immune-privilege. *Sci. Rep.* 2019, 9, 15919. [CrossRef] [PubMed]
54. Mezrich, J.D.; Fechner, J.H.; Zhang, X.; Johnson, B.P.; Burlington, W.J.; Bradfield, C.A. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J. Immunol.* 2010, 185, 3190–3198. [CrossRef]
55. Pallotta, M.T.; Orabona, C.; Volpi, C.; Vacca, C.; Belladonna, M.L.; Bianchi, R.; Servillo, G.; Brunacci, C.; Calvitti, M.; Bicciato, S.; et al. Role of indoleamine 2,3-dioxygenase in testicular immune privilege. *Autoimmun. Rev.* 2011, 107–112. [CrossRef]
56. Kekäläinen, E.; Tuovinen, H.; Joensuu, J.; Gylling, M.; Franssila, R.; Pöntynen, N.; Talvensaari, K.; Perheentupa, J.; Miettinen, A.; Arstila, T.P. A defect of regulatory T cells in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *Dtsch. Ärzteblatt Int.* 2013, 110, 339–346. [CrossRef] [PubMed]
62. Jacobo, P.; Guazzzone, V.A.; Jarazo-Dietrich, S.; Theas, M.S.; Lustig, L. Differential changes in CD4+ and CD8+ effector and regulatory T lymphocyte subsets in the testis of rats undergoing autoimmune orchitis. *J. Reprod. Immunol.* 2009, 81, 44–54. [CrossRef] [PubMed]

63. Gong, J.; Zeng, Q.; Yu, D.; Duan, Y.G. T Lymphocytes and Testicular Immunity: A New Insight into Immune Regulation in Testes. *Int. J. Mol. Sci.* 2020, 22, 57. [CrossRef] [PubMed]

64. Bhati, M.; Llamosas, E.; Jacques, D.A.; Jeffries, C.M.; Dastmalchi, S.; Ripin, N.; Nicholas, H.R.; Matthews, J.M. Interactions between LH3X- and ISL1-family LIM-homeodomain transcription factors are conserved in Caenorhabditis elegans. *Sci. Rep.* 2017, 7, 4579. [CrossRef]

65. Bhushan, S.; Theas, M.S.; Guazzzone, V.A.; Jacobo, P.; Wang, M.; Fijak, M.; Meinhardt, A.; Lustig, L. Immune Cell Subtypes and Their Function in the Testis. *Front. Immunol.* 2020, 11, 583304. [CrossRef]

66. Hedger, M.P.; Meinhardt, A. Local regulation of T cell numbers and lymphocyte-inhibiting activity in the interstitial tissue of the adult rat testis. *J. Reprod. Immunol.* 2000, 47, 69–80. [CrossRef]

67. Dai, Z.; Nasr, I.W.; Reel, M.; Deng, S.; Diggs, L.; Larsen, C.P.; Rothstein, D.M.; Lakks, F.G. Impaired recall of CD8 memory T cells in immunologically privileged tissue. *J. Immunol. 2005, 174, 1165–1170. [CrossRef]

68. Nasr, I.W.; Wang, Y.; Gao, G.; Deng, S.; Diggs, L.; Rothstein, D.M.; Tellides, G.; Lakks, F.G.; Dai, Z. Testicular immune privilege promotes transplantation tolerance by altering the balance between memory and regulatory T cells. *J. Immunol. 2005, 174, 1611–1618. [CrossRef]

69. Cheng, X.; Dai, H.; Wan, N.; Moore, Y.; Vankayalapati, R.; Dai, Z. Interaction of programmed death-1 and programmed death-1 ligand-1 contributes to testicular immune privilege. *Transplantation 2009, 87, 1778–1786. [CrossRef]

70. Zhou, R.; Wu, J.; Liu, B.; Jiang, Y.; Chen, W.; Li, J.; He, Q.; He, Z. The roles and mechanisms of Leydig cells and myoid cells in regulating spermatogenesis. *Cell. Mol. Life Sci.* 2019, 76, 2681–2695. [CrossRef]

71. Jahnukainen, K.; Saari, T.; Salmi, T.T.; Pöllänen, P.; Pellinimi, L.J. Reactions of Leydig cells and blood vessels to lymphoblastic leukemia in the rat testis. *Leukemia 1995, 9, 69–90. [PubMed]

72. Sainio-Pöllänen, S.; Sundström, J.; Erkkilä, S.; Hänninen, A.; Vainiopää, M.; Martikainen, M.; Salminen, E.; Veräjänkorva, E.; Antola, H.; Nikula, H.; et al. CD106 (VCAM-1) in testicular immunoregulation. *J. Reprod. Immunol.* 1997, 33, 221–238. [CrossRef]

73. Dutta, D.; Park, I.; Guililat, H.; Sang, S.; Talapatra, A.; Hansen, L.; Mills, N.C. Ethylene dimethane sulfonate (EDS) ablation of Leydig cells in adult rat depletes testosterone resulting in epididymal sperm granuloma: Testosterone replacement prevents granuloma formation. *Reprod. Biol. 2019, 19, 89–99. [CrossRef] [PubMed]

74. Fijak, M.; Schneider, E.; Klug, J.; Bhushan, S.; Hackstein, H.; Schuler, G.; Wygrecka, M.; Gromoll, J.; Meinhardt, A. Testosterone replacement effectively inhibits the development of experimental autoimmune orchitis in rats: Evidence for a direct role of testosterone on regulatory T cell expansion. *J. Immunol. 2011, 186, 5162–5172. [CrossRef] [PubMed]

75. Monsivais, D.; Matzuk, M.M.; Pangas, S.A. The TGF-β Family in the Reproductive Tract. *Cold Spring Harb. Perspect. Biol. 2017, 9, a022251. [CrossRef] [PubMed]

76. Dal Secco, V.; Riccioli, A.; Padula, F.; Ziparo, E.; Filippini, A. Mouse Sertoli cells display phenotypical and functional traits of antigen-presenting cells in response to interferon gamma. *Biol. Reprod. 2008, 78, 234–242. [CrossRef]

77. Fallarino, F.; Luca, G.; Calvitti, M.; Mancuso, F.; Nastruzzi, C.; Fioretti, M.C.; Grohmann, U.; Becchetti, E.; Burgevin, A.; Kratzer, R.; et al. Therapy of experimental type 1 diabetes by isolated Sertoli cell xenografts alone. *Reprod. Biol. 2005, 2, 2511–2526. [CrossRef]

78. 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]

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

80. Yang, H.; Böttner, A.; Albiol, L.; Julien, C.; Thiele, T.; Figge, C.; Kramer, I.; Kneissel, M.; Duda, G.N.; Checa, S.; et al. Cortical bone adaptation to a moderate level of mechanical loading in male Sost deficient mice. *Sci. Rep. 2020, 10, 22299. [CrossRef]

81. Lundy, S.D.; Sangwan, N.; Parekh, N.V.; Selvam, M.K.P.; Gupta, S.; McCaffrey, P.; Besoff, K.; Vlah, 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] [PubMed]

82. Wilharm, A.; Briggs, H.C.; Sandrock, I.; Ribeiro, M.; Amado, T.; Reinhardt, A.; Demera, A.; Hoenicke, L.; Strowiag, T.; Carvalho, T.; et al. Microbiota-dependent establishment of testicular IL-17-producing Vγ6Vγ7 T cells upon puberty promotes local tissue immune surveillance. *Mucosal Immunol. 2021, 14, 242–252. [CrossRef] [PubMed]

83. Gachet, C.; Prat, M.; Burucoa, C.; Grivard, P.; Pichon, M. Spermatic Microbiome Characteristics in Infertile Patients: Impact on Sperm Count, Mobility, and *J. Clin. Med. 2022, 11, 1505. [CrossRef] [PubMed]

84. Charitos, I.A.; Topi, S.; Gagliano-Candela, R.; De Nitto, E.; Polimeno, L.; Montagnani, M.; Santacroce, L. The toxic effects of endocrine disrupting chemicals (EDCs) on gut microbiota: Bisphenol A (BPA). A review. *Endocr. Metab. Immune Disord. Drug Targets 2022, 22, 716–727. [CrossRef]

85. Santacroce, L.; Bottalcio, L.; Topi, S.; Castellaneta, F.; Charitos, I.A. The “Scourge of the Renaissance”. A Short Review about Treponema pallidum infection. *Endocr. Metab. Immune Disord. Drug Targets 2020, 20, 335–343. [CrossRef]
[111. Schirinzi, A.; Cazzolla, A.P.; Mascolo, E.; Palmieri, G.; Pesce, F.; Gesualdo, L.; Santacroce, L.; Ballini, A.; Lovero, R.; Di Serio, F. Determination of the Upper Reference Limit of Human Epididymis Secretory Protein 4 (HE4) in Healthy Male Individuals and Correlation with Renal and Fertility Markers. Endocr. Metab. Immune Disord. Drug Targets 2021, 21, 912–918. [CrossRef]

112. Stammier, A.; Hau, T.; Bhushan, S.; Meinhardt, A.; Jonigk, D.; Lippmann, T.; Plätz, A.; Schneider-Hüther, I.; Middendorff, R. Epididymitis: Ascending infection restricted by segmental infection. Hum. Reprod. 2015, 30, 1557–1563. [CrossRef]

113. Silva, E.J.R.; Ribeiro, C.M.; Mirim, A.F.M.; Silva, A.A.S.; Romano, R.M.; Hallak, J.; Avellar, M.C.W. Lipopolysaccharide and lipotheicoic acid differentially modulate epididyimal cytokine and chemokine profiles and sperm parameters in experimental acute epididymitis. Sci. Rep. 2018, 8, 103. [CrossRef][PubMed]

114. Mutoji, K.; Singh, A.; Nguyen, T.; Gildersleeve, H.; Kaucher, A.V.; Oatley, M.J.; Oatley, J.M.; Velte, E.K.; Geyer, C.B.; Cheng, K.; et al. TSPAN8 Expression Distinguishes Spermatogonial Stem Cells in the Prepubertal Mouse Testis. Biol. Reprod. 2016, 95, 117. [CrossRef][PubMed]

115. Biswas, B.; Bhushan, S.; Rajesh, A.; Suraj, S.K.; Lu, Y.; Meinhardt, A.; Yenugu, S. Uropathogenic Escherichia coli (UPEC) induced antimicrobial gene expression in the male reproductive tract of rat: Evaluation of the potential of Defensin 21 to limit infection. Andrology 2015, 3, 368–375. [CrossRef][PubMed]

116. Bryan, E.R.; Kollipara, A.; Trim, L.K.; Armitage, C.W.; Carey, A.J.; Mihalas, B.; Redgrove, K.A.; Mclaughlin, E.A.; Beagley, K.W. Hematogenous dissemination of Chlamydia muridurum from the urethra in macrophages causes testicular infection and sperm DNA damage. Biol. Reprod. 2019, 101, 748–759. [CrossRef][PubMed]

117. Emerson, C.; Dinsmore, W.W.; Quah, S.P. Are we missing mumps epididymo-orchitis? Int. J. STD AIDS 2007, 18, 341–342. [CrossRef]

118. Giagulli, V.A.; Guastamacchia, E.; Magrone, T.; Jirillo, E.; Lisco, G.; De Pergola, G.; Triggiani, V. Worse progression of COVID-19 in men: Is testosterone a key factor? Andrology 2021, 9, 53–64. [CrossRef]

119. Santacroce, L.; Charitos, I.A.; Carretta, D.M.; De Nitto, E.; Lovero, R. The human coronaviruses (HCoVs) and the molecular mechanisms of SARS-CoV-2 infection. J. Mol. Med. 2021, 99, 93–106. [CrossRef]

120. Schirinzi, A.; Cazzolla, A.P.; Lovero, R.; Lo Muzio, L.; Testa, N.F.; Ciavarella, D.; Palmieri, G.; Pozzessere, P.; Proacci, V.; Di Serio, F.; et al. New Insights in Laboratory Testing for COVID-19 Patients: Looking for the Role and Predictive Value of Human epididymis secretory protein 4 (HE4) and the Innate Immunity of the Oral Cavity and Respiratory Tract. Microorganisms 2020, 8, 1718. [CrossRef]

121. Vabret, N.; Britton, G.J.; Gruber, C.; Hegde, S.; Chung, R.; Mihalas, B.; Redgrove, K.A.; Mclaughlin, E.A.; Beagley, K.W. Immunology of COVID-19: Current State of the Science. Immunity 2020, 52, 910–941. [CrossRef][PubMed]

122. Yang, M.; Chen, S.; Huang, B.; Zhong, J.M.; Su, H.; Chen, Y.J.; Cao, Q.; Ma, L.; He, J.; Li, X.F.; et al. Pathological Findings in the Testes of COVID-19 Patients: Clinical Implications. Eur. Urol. Focus 2020, 6, 1124–1129. [CrossRef]

123. Fermanosra, A.; Zara, V. Diet and Male Fertility: The Impact of Nutrients and Antioxidants on Sperm Energetic Metabolism. Int. J. Mol. Sci. 2022, 23, 2542. [CrossRef]

124. Suliga, E.; Gluszek, S. The relationship between diet, energy balance and fertility in men. Int. J. Vitam. Nutr. Res. 2020, 90, 514–526. [CrossRef][PubMed]

125. Guasch-Ferré, M.; Willett, W.C. The Mediterranean diet and health: A comprehensive overview. J. Intern. Med. 2021, 290, 549–566. [CrossRef][PubMed]

126. Bachir, B.G.; Jarvi, K. Infectious, inflammatory, and immunologic conditions resulting in male infertility. Urol. Clin. N. Am. 2014, 41, 67–81. [CrossRef]

127. Morielli, T.; O’Flaherty, C. Oxidative stress impairs function and increases redox protein modifications in human spermatozoa. Reproduction 2015, 149, 113–123. [CrossRef]

128. Fermanosra, A.; Conte, A.; Moscatelli, N.; Zara, V. A high-fat diet negatively affects rat sperm mitochondrial respiration. Andrology 2016, 4, 520–525. [CrossRef]

129. Cases, R.; Estruch, R.; Sacanella, E. The Protective Effects of Extra Virgin Olive Oil on Immune-mediated Inflammatory Responses. Endocr. Metab. Immune Disord. Drug Targets 2018, 18, 23–35. [CrossRef]

130. Magrone, T.; Spagnolletta, A.; Salvatore, R.; Magrone, M.; Dentamaro, F.; Russo, M.A.; Difonzo, G.; Summo, C.; Caponio, F.; Jirillo, E. Olive Leaf Extracts Act as Modulators of the Human Immune Response. J. Mol. Sci. 2022, 5, 2542. [CrossRef]

131. Visser, B.; Heitmeijer, L.; Sluijs, I.; van der Klis, F.; de Vries, J.; van der Schouw, Y.; Luijckx, G.; Verschoor, P.; van der Kuip, D.; Nieuwenhuijs, B.; Katan, M.; van der Schouw, Y.; Health Protection Agency. The Mediterranean diet and health: A comprehensive overview. J. Intern. Med. 2021, 290, 549–566. [CrossRef][PubMed]

132. Bachir, B.G.; Jarvi, K. Infectious, inflammatory, and immunologic conditions resulting in male infertility. Urol. Clin. N. Am. 2014, 41, 67–81. [CrossRef]

133. Morielli, T.; O’Flaherty, C. Oxidative stress impairs function and increases redox protein modifications in human spermatozoa. Reproduction 2015, 149, 113–123. [CrossRef]

134. Fermanosra, A.; Conte, A.; Moscatelli, N.; Zara, V. A high-fat diet negatively affects rat sperm mitochondrial respiration. Andrology 2016, 4, 520–525. [CrossRef]

135. Cases, R.; Estruch, R.; Sacanella, E. The Protective Effects of Extra Virgin Olive Oil on Immune-mediated Inflammatory Responses. Endocr. Metab. Immune Disord. Drug Targets 2018, 18, 23–35. [CrossRef]

136. Magrone, T.; Spagnolletta, A.; Salvatore, R.; Magrone, M.; Dentamaro, F.; Russo, M.A.; Difonzo, G.; Summo, C.; Caponio, F.; Jirillo, E. Olive Leaf Extracts Act as Modulators of the Human Immune Response. J. Mol. Sci. 2022, 5, 2542. [CrossRef]

137. Muller, A.K.; Albrecht, F.; Rohrer, C.; Koeberle, A.; Werz, O.; Schöllmann, W.; Glei, M.; Lorkowski, S.; Wallert, M. Olive Oil Extracts and Oleic Acid Attenuate the LPS-Induced Inflammatory Response in Murine RAW264.7 Macrophages but Induce the Release of Prostaglandin E2. Nutrients. 2021, 13, 4437. [CrossRef][PubMed]

138. Santacroce, L.; Incingolo, F.; Topi, S.; Del Prete, R.; Di Cosola, M.; Charitos, I.A.; Montagnani, M. Potential beneficial role of probiotics on the outcome of COVID-19 patients: An evolving perspective. Diabetes Metab. Syndr. 2021, 15, 295–301. [CrossRef][PubMed]

139. Jones, S.E.; Versalovic, J. Probiotic Lactobacillus reuteri biofilms produce antimicrobial and anti-inflammatory factors. BMC Microbiol. 2009, 9, 35. [CrossRef][PubMed]
135. Yan, F.; Cao, H.; Cover, T.L.; Whitehead, R.; Washington, M.K.; Polk, D.B. Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. *Gastroenterology* **2007**, *132*, 562–575. [CrossRef]

136. Valcarce, D.G.; Genovès, R.; Riesco, M.F.; Martorell, P.; Herráez, M.P.; Ramón, D.; Robles, V. Probiotic administration improves sperm quality in asthenozoospermic human donors. *Benef. Microbes* **2017**, *8*, 193–206. [CrossRef]

137. Pacifici, L.; Santacroce, L.; Di Domenico, M.; Ballini, A.; Boccellino, M.; Lovero, R.; Santacroce, L. The Intestinal Microbiota May Be a Potential Theranostic Tool for Personalized Medicine. *J. Pers. Med.* **2022**, *12*, e0185964. [CrossRef]

138. Ibrahim, M.A.A.; Abdeljawaad, K.A.A.; Abdelrahman, A.H.M.; Jaragh-Alhadad, L.A.; Oraby, H.F.; Abulhassan, H.; Robles, S.; Abd El-Aziz, M.; El-Sayed, A.; et al. Male Oxidative Stress Infertility (MOSI): Proposed Terminology and Clinical Practice Guidelines for Management of Idiopathic Male Infertility. *World J. Men’s Health* **2019**, *37*, 296–312. [CrossRef] [PubMed]

139. Aly, H.A.; El-Beshbishy, H.A.; Banjar, Z.M. Mitochondrial dysfunction induced impairment of spermatogenesis in LPS-treated rats: Modulatory role of lycopene. *Eur. J. Pharmacol.* **2012**, *677*, 31–38. [CrossRef] [PubMed]

140. Schisterman, E.F.; Sjaarda, L.A.; Clemons, T.; Cantore, S.; Altini, V.; Pacifici, A.; De Vito, D.; Pettini, F.; et al. Gender medicine: The impact of probiotics on male patients. *Clin. Ter.* **2021**, *171*, e8–e15. [CrossRef]

141. Magrone, T.; Magrone, M.; Russo, M.A.; Jirillo, E. Recent Advances on the Anti-Inflammatory and Antioxidant Properties of Red Grape Polyphenols: In Vitro and In Vivo Studies. *Antioxidants* **2019**, *8*, 35. [CrossRef]

142. Agarwal, A.; Parekh, N.; Panner Selvam, M.K.; Henkel, R.; Shah, R.; Homa, S.T.; Ramasamy, R.; Ko, E.; Tremellen, K.; Esteves, S.; et al. Male Oxidative Stress Infertility (MOSI): Proposed Terminology and Clinical Practice Guidelines for Management of Idiopathic Male Infertility. *World J. Men’s Health* **2019**, *37*, 296–312. [CrossRef] [PubMed]

143. Ibrahim, M.A.A.; Abdeljawaad, K.A.A.; Abdelrahman, A.H.M.; Jaragh-Alhadad, L.A.; Oraby, H.F.; El-Sayed, A.; et al. Male Oxidative Stress Infertility (MOSI): Proposed Terminology and Clinical Practice Guidelines for Management of Idiopathic Male Infertility. *World J. Men’s Health* **2019**, *37*, 296–312. [CrossRef] [PubMed]

144. Magrone, T.; Jirillo, E. Recent Advances on the Anti-Inflammatory and Antioxidant Properties of Red Grape Polyphenols: In Vitro and In Vivo Studies. *Antioxidants* **2019**, *8*, 35. [CrossRef]

145. Aly, H.A.; El-Beshbishy, H.A.; Banjar, Z.M. Mitochondrial dysfunction induced impairment of spermatogenesis in LPS-treated rats: Modulatory role of lycopene. *Eur. J. Pharmacol.* **2012**, *677*, 31–38. [CrossRef] [PubMed]

146. Schisterman, E.F.; Sjaarda, L.A.; Clemons, T.; Cantore, S.; Altini, V.; Pacifici, A.; De Vito, D.; Pettini, F.; et al. Gender medicine: The impact of probiotics on male patients. *Clin. Ter.* **2021**, *171*, e8–e15. [CrossRef]

147. Magrone, T.; Magrone, M.; Russo, M.A.; Jirillo, E. Recent Advances on the Anti-Inflammatory and Antioxidant Properties of Red Grape Polyphenols: In Vitro and In Vivo Studies. *Antioxidants* **2019**, *8*, 35. [CrossRef]

148. Magrone, T.; Jirillo, E. Recent Advances on the Anti-Inflammatory and Antioxidant Properties of Red Grape Polyphenols: In Vitro and In Vivo Studies. *Antioxidants* **2019**, *8*, 35. [CrossRef]

149. Ibrahim, M.A.A.; Abdeljawaad, K.A.A.; Abdelrahman, A.H.M.; Jaragh-Alhadad, L.A.; Oraby, H.F.; El-Sayed, A.; et al. Male Oxidative Stress Infertility (MOSI): Proposed Terminology and Clinical Practice Guidelines for Management of Idiopathic Male Infertility. *World J. Men’s Health* **2019**, *37*, 296–312. [CrossRef] [PubMed]

150. Ibrahim, M.A.A.; Abdeljawaad, K.A.A.; Abdelrahman, A.H.M.; Jaragh-Alhadad, L.A.; Oraby, H.F.; El-Sayed, A.; et al. Male Oxidative Stress Infertility (MOSI): Proposed Terminology and Clinical Practice Guidelines for Management of Idiopathic Male Infertility. *World J. Men’s Health* **2019**, *37*, 296–312. [CrossRef] [PubMed]

151. Ibrahim, M.A.A.; Abdeljawaad, K.A.A.; Abdelrahman, A.H.M.; Jaragh-Alhadad, L.A.; Oraby, H.F.; El-Sayed, A.; et al. Male Oxidative Stress Infertility (MOSI): Proposed Terminology and Clinical Practice Guidelines for Management of Idiopathic Male Infertility. *World J. Men’s Health* **2019**, *37*, 296–312. [CrossRef] [PubMed]

152. Ibrahim, M.A.A.; Abdeljawaad, K.A.A.; Abdelrahman, A.H.M.; Jaragh-Alhadad, L.A.; Oraby, H.F.; El-Sayed, A.; et al. Male Oxidative Stress Infertility (MOSI): Proposed Terminology and Clinical Practice Guidelines for Management of Idiopathic Male Infertility. *World J. Men’s Health* **2019**, *37*, 296–312. [CrossRef] [PubMed]

153. Ibrahim, M.A.A.; Abdeljawaad, K.A.A.; Abdelrahman, A.H.M.; Jaragh-Alhadad, L.A.; Oraby, H.F.; El-Sayed, A.; et al. Male Oxidative Stress Infertility (MOSI): Proposed Terminology and Clinical Practice Guidelines for Management of Idiopathic Male Infertility. *World J. Men’s Health* **2019**, *37*, 296–312. [CrossRef] [PubMed]