Observational Study

Medicine

OPEN

Gut microbiota and male fertility

A two-sample Mendelian randomization study

Zhi-hong Wang, MS[a], Yi-fan Kang, PhD[b,*]

Abstract

Previous studies have reported that alterations in gut microbiota composition are associated with male fertility. However, it is unclear and difficult to establish whether these associations reflect a causal relationship. We assessed genome-wide association study summary statistics for gut microbiota and male fertility to perform MR analysis. Independent single nucleotide polymorphisms closely associated with 211 gut bacterial taxa (N = 122,110) were identified as instrumental variables. The summary statistic data for male infertility (N = 733,479), abnormal spermatozoa (N = 209,921) and erectile dysfunction (N = 223,805) were obtained from the latest release from the FinnGen consortium as the outcome of interest. Two-sample MR was performed to evaluate the causal effect of gut microbiota on male fertility, including inverse-variance-weighted (IVW) method, weighted median method, MR-Egger, mode-based estimation and MR-PRESSO. A series of sensitivity analyses was performed to validate the robustness of the results. The robustness of the estimation was tested by a series of sensitivity analyses including Cochran's Q test, MR-Egger intercept analysis, leave-one-out analysis and funnel plot were used to assess the causal association. Combining the results from the discovery and replication stages, we identified 3 causal bacterial genus. Ruminiclostridm6 (OR = 0.537, 95%CI = 0.292–0.987, *P* = .045, PFDR = 0.234) was found to be closely associated with male infertility, and the decrease in its quantity increased the risk of male infertility. Decreased Prevotella9 (OR = 0.670, 95% CI = 0.452–0.992, *P* = .046, PFDR = 0.175) was found to be closely related to abnormal sperm. Lachnospiraceae NC2004 group (OR = 1.173, 95% CI = 1.008–1.366, *P* = .078, PFDR = 0.530) was found to be closely related to male erectile dysfunction, and there was a positive correlation between them. No heterogeneity and pleiotropy were detected. This study implied a causal relationship between the Ruminiclostridm6 genus, Prevotella9 genus, Lachnospiraceae NC2004 group genus and male fertility, thus providing novel insights into the gut microbiota-mediated development mechanism of ADs. Nevertheless, future studies are warranted to dissect the underlying mechanisms of specific bacterial taxa's role in the pathophysiology of male fertility.

Abbreviations: GWAS = genome-wide association study, IVs = instrumental variables, IVW = inverse-variance-weighted, LD = linkage disequilibrium, MR = Mendelian randomization, MR-PRESSO = MR Pleiotropy residual and outlier method, OR = odds ratio, TMAO = trimethylamino oxide.

Keywords: causality, gut microbiota, male fertility, Mendelian randomization

1. Introduction

It is estimated that about 8% to 12% of couples in the world are unable to have a normal pregnancy due to infertility, among which male factors account for about 50%.[1] The causes of male infertility are various, mostly due to genetic factors.[2] At present, environmental, work-and-rest, diet, and other causes of male fertility problems have gradually taken over the majority of the causes.

The gut microbiota has received increasing attention over the past decade. Human intestinal microflora is divided into 4 main phyla, including Bacillota, Bacteroidetes, Actinobacteria and Proteobacteria.[3] Among them, Bacillota and Bacteroidetes account for 90% of the intestinal microbiota.[4] Microbial dysbiosis is now recognized as an important cause or exacerbator of many human disease states and is found to be closely related to a variety of diseases.[5,6] Among them, fecal microbiota transplantation is a key new treatment method, which can restore the intestinal microbiota to the pre-disease state.[7] Intestinal microbiota transplanted into animals can promote spermatogenesis and improve semen quality.[8] It has been established that the gut microbiota plays a crucial role in spermatogenesis and in improving male fertility.[9] However, the literature exploring the role of the microbiota in male infertility remains sparse.

The authors have no funding and conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

No additional ethical approval is required as this is a re-analysis of data that is already publicly available.

[a] Department of Reproductive Center, The First Hospital of Shanxi Medical University, Taiyuan, China, [b] The Fifth Clinical Medical College of Shanxi Medical University, Shanxi Provincial People's Hospital, Taiyuan, China.

** Correspondence: Yi-fan Kang, Department of Reproductive Center, Shanxi Medical University, The Fifth Hospital of Shanxi Medical University, 29 Shuangta East Street, Yingze District, Taiyuan City, Shanxi Province 030001, China (e-mail: 384078163@qq.com).*

How to cite this article: Wang Z-h, Kang Y-f. Gut microbiota and male fertility: A two-sample Mendelian randomization study. Medicine 2025;104:8(e41542).

Received: 14 October 2024 / Received in final form: 25 January 2025 / Accepted: 28 January 2025

http://dx.doi.org/10.1097/MD.0000000000041542

Mendelian randomization (MR) analysis is an effective method that uses genetic variants as instrumental variables (IVs) to assess the potential causal relationship between a specific risk factor and a disease of interest. MR has been widely used to explore the causal relationship between gut microbiota and diseases, including metabolic diseases, inflammatory diseases, and rheumatological diseases. Previous Mendelian randomization studies have investigated the relationship between gut microbiota and infertility in women,[10] but not in men. Alterations in the gut microbiota have been found in men with infertility, abnormal sperm and erectile dysfunction. Causal inference between gut microbiota and male infertility is limited by inconsistent results from single-observation studies and the possibility of confounding and reverse causality.

Therefore, in this study, a two-sample MR Analysis was performed to assess the causal relationship between gut microbiota and male fertility using the genome-wide association study (GWAS) summary statistics from the MiBioGen and FinnGen consortium.

2. Methods

All data used in this MR Study are publicly available. GWAS data of gut microbiota were obtained from MiBioGen International consortium data. Pooled GWAS data with phenotypes of male infertility, abnormal sperm, and erectile dysfunction were obtained from FinnGen. We accessed data on 5 gut microbial taxa at 211 taxonomic levels. The study populations were predominantly European, with total samples of 733,479, 209,921, and 223,805 persons, respectively, corresponding to male infertility, abnormal sperm, and erectile dysfunction.

Existing ethical clearance from each institutional review board applies to all published genomewide association studies (GWAS). Only abstract-level data were used in this study and no additional ethical approval was required.

All MR analysis using R software (version 4.2.2) through the "Two sample MR" package (https://mrcieu.github.io/TwoSampleMR). $P < .05$ was accepted as statistically significant for the MR analysis. This study used a two-sample MR design with SNPs as instrumental variables to assess the causal interaction between gut microbiota and male fertility. SNPs were required to meet 3 criteria: being highly associated with exposure under study; it was not associated with any other confounding factors; and it only affects the outcome through exposure, and has nothing to do with the outcome itself.[11] Figure 1 illustrates a schematic of the study design. We selected SNPs that reached the genome-wide significance threshold ($P < 5 \times 10^{-8}$) were used as instrumental variables (IV). Because the number of SNPS meeting the criteria was too small, we chose the threshold of $P < 1 \times 10^{-5}$IV. To obtain more comprehensive results. To ensure the independence of each SNP, linkage disequilibrium (LD) factor $r^2 < 0.0001$ and clustering window width kb = 10,000 kb were set. In addition, this study searched the Phenoscanner database for all phenotypes associated with instrumental variables, and no SNPS were found to be associated with confounding factors associated with outcomes.

We used inverse variance weighting (IVW), weighted median, and MR-Egger for MR Analysis of gut microbial taxa with multiple IVs. The fixed or stochastic effect IVW method is used as the primary method for MR analysis. Cochrane's Q test was performed to assess the heterogeneity of IV if there was heterogeneity ($P < .05$), the random effects IVW test was used to estimate the MR statistic, otherwise the fixed effects model was used. Horizontal pleiotropy was used to balance the effects. The MR-Egger truncation term is one of the most important methods to assess the presence of horizontal pleiotropy between IVs. When the intercept is nonzero, there is horizontal polymorphism, whose statistical significance will be determined by the P value of the intercept. Together with MR-Egger, the MR Pleiotropy residual and outlier method (MR-PRESSO) is also commonly used to detect horizontal pleiotropy. MR-PRESSO consists of the following 3 main steps: detect the presence of pleiotropy, calculate the corrected prediction after removing the outliers, and compare whether the difference between the 2 predictions before and after correction is statistically significant. Finally, a leave-one-out analysis is used to assess the robustness of the MR results.

3. Results

In the genetic analysis of gut microbiota, by removing SNPs with linkage disequilibrium and palindromic structures with full locus significance level, a total of 2774 SNPs were used as instrumental variables for 211 bacteria according to the selection criteria of instrumental variables, with $P < 1 \times 10^{-5}$ as the threshold. These included 9 phyla (108 SNPS), 16 classes (194 SNPS), 20 orders (237 SNPS), 35 families (427 SNPS), and 131 genera (1461 SNPS). F-statistics were calculated for each SNP and all were larger than a threshold of 10. In the heterogeneity test, Cochran's Q statistic was all greater than 0.05, indicating no heterogeneity among SNPs; Therefore, the fixed effect IVW model was used as the main analysis method in the MR analysis. Table 1 enumerates the significant strain results for the 5 MR statistical methods.

Combining the results from the discovery and replication stages, we identified 3 causal bacterial genus. The odds ratio (OR) is calculated as the ratio of the odds of exposure in

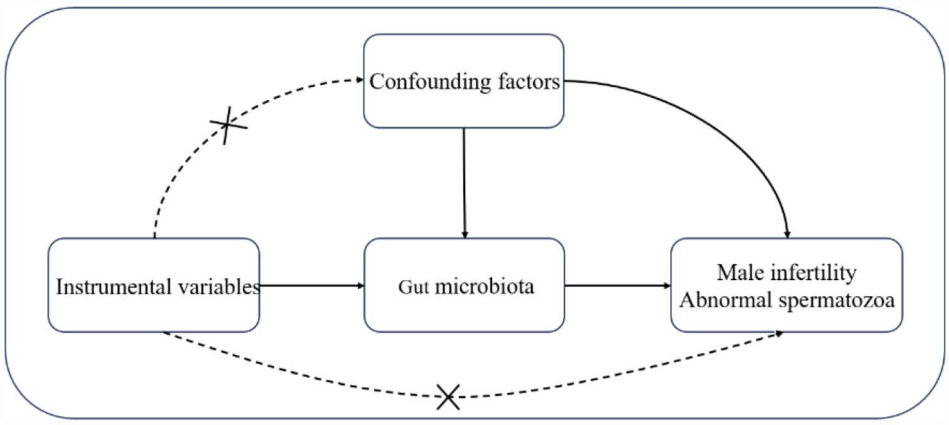

Figure 1. Three key assumptions for a valid Mendelian randomization study.

Table 1

Significant and nominally significant Mendelian randomization estimates of the associations from Firmicutes on male infertility, abnormal spermatoz and erectile dysfunction

								Heterogeneity		Horizontal pleiotropy		
Exposure	Outcome	MR method	nSNPs	Beta	SE	*P* value	OR	95%CI	Cochran's Q	*P* value	Egger intercept	SE	*P* value
Genus Ruminiclostridm6 052	Male infertility	MR Egger	13	−1.608	1.026	.145	0.200	(0.027, 1.497)	13.862	.241	0.076	0.075	.335
		Weighted median	13	−0.600	0.393	.127	0.549	(0.254, 1.186)					
		Inverse variance weighted	13	−0.622	0.311	.045	0.537	(0.292, 0.987)	15.142	.234			
		Simple mode	13	−0.445	0.712	.544	0.641	(0.159, 2.589)					
		Weighted mode	13	−0.613	0.637	.355	0.542	(0.156, 1.887)					
Genus prevotella9 045	Abnormal spermatoz	MR Egger	15	−0.449	0.608	.473	0.639	(0.194, 2.101)	18.742	.131	0.005	0.060	.935
		Weighted median	15	−0.398	0.247	.107	0.672	(0.414, 1.089)					
		Inverse variance weighted	15	−0.401	0.201	.046	0.670	(0.452, 0.992)	18.752	.175			
		Simple mode	15	−0.473	0.388	.243	0.623	(0.291, 1.334)					
		Weighted mode	15	−0.451	0.330	.194	0.637	(0.333, 1.217)					
Genus Lachnospiraceae NC2004 group 022	Erectile dysfunction	MR Egger	9	0.409	0.321	.243	1.505	(0.803, 2.823)	6.509	.482	−0.023	0.024	.348
		Weighted median	9	0.204	0.104	.049	1.227	(1.001, 1.504)					
		Inverse variance weighted	9	0.160	0.078	.039	1.173	(1.008, 1.366)	7.151	.520			
		Simple mode	9	0.302	0.167	.109	1.352	(0.974, 1.877)					
		Weighted mode	9	0.291	0.177	.140	1.337	(0.945, 1.892)					

the case group to the odds of exposure in the control group. Specifically, it is the ratio of the proportion of exposed individuals to non-exposed individuals in the case group divided by the corresponding ratio in the control group. When OR is greater than 1, it suggests that the factor under investigation is a risk factor, indicating a higher likelihood of disease occurrence among those exposed. Conversely, when OR is less than 1, it implies that the factor may serve as a protective factor, suggesting a reduced likelihood of disease occurrence among those exposed. Ruminiclostridm6 (OR = 0.537, 95% CI = 0.292–0.987, *P* = .045, PFDR = 0.234) was found to be closely associated with male infertility, and the decrease in its quantity increased the risk of male infertility. Decreased Prevotella9 (OR = 0.670, 95% CI = 0.452–0.992, *P* = .046, PFDR = 0.175) was found to be closely related to abnormal sperm. Lachnospiraceae (OR = 1.173, 95% CI = 1.008–1.366, *P* = .078, PFDR = 0.530) was found to be closely related to male erectile dysfunction, and there was a positive correlation between them. No heterogeneity and pleiotropy were detected. Figure 2 showed scatter plots of the causal effects of the significant microbial taxa on male infertility, abnormal spermatozoa and erectile dysfunction.

After the leave-one-out analysis, the overall error line changes little after excluding each SNP (Fig. 3), and the funnel plot shows that the points of the left and right distributions are essentially symmetric, indicating that the results are stable (Fig. 4). In addition, MR Regression analysis showed the significance of the results (Fig. 5).

4. Discussion

In this study, we performed a two-sample MR analysis to investigate the causal relationship between male fertility and gut microbiota. The results of this study indicate that Ruminiclostridm6, Prevotella9 and Lachnospiraceae NC2004 group are key gut bacterial species that affect male infertility, abnormal sperm and erectile dysfunction, respectively.

There are various causes of male infertility, and the fundamental cause is that the sperm production process is blocked, resulting in no sperm or mature sperm production.[12] Disruption of microbiota composition or function and subsequent altered host-microbiota interactions perturb the stability of multiple organ systems, including the body,[13] while also affecting spermatogenesis.[14] Intestinal flora can act on the male reproductive system through trace elements, metabolism, inflammatory response, and nerve function,[15] which is potentially related to male infertility.

Various trace elements in the body can cause abnormalities in sperm. Abnormal gut microbiota and hormone synthesis caused by iron deficiency can indirectly impair spermatogenesis.[16] At the same time, sperm motility needs to increase the concentration of intracellular calcium ions.[17] Hadwan et al[18] found that semen volume and sperm motility of infertile men with asthenospermia increased significantly after 3 months of daily zinc supplementation at 220 mg. The regulation of intestinal microbiota will change the level of bile acids and further affect the absorption of vitamin A by the host intestine, leading to impaired spermatogenesis.[19] It has been proved that vitamin D supplementation can improve serum 25-OH-Vitamin D_3 levels, and become a potential effective method for the treatment or prevention of male semen quality.[20] Various trace elements are involved in the function of the various organs of the human body and can affect male fertility.

The gut microbiota can mediate the metabolite to affect insulin resistance and further indirectly regulate male fertility. Studies have shown that insulin resistance index is negatively correlated with semen volume and the percentage of progressively motile sperm.[21,22] In addition, spermine is used to maintain normal reproductive physiology[23] and can be synthesized by endogenous polyamine metabolism or obtained from dietary

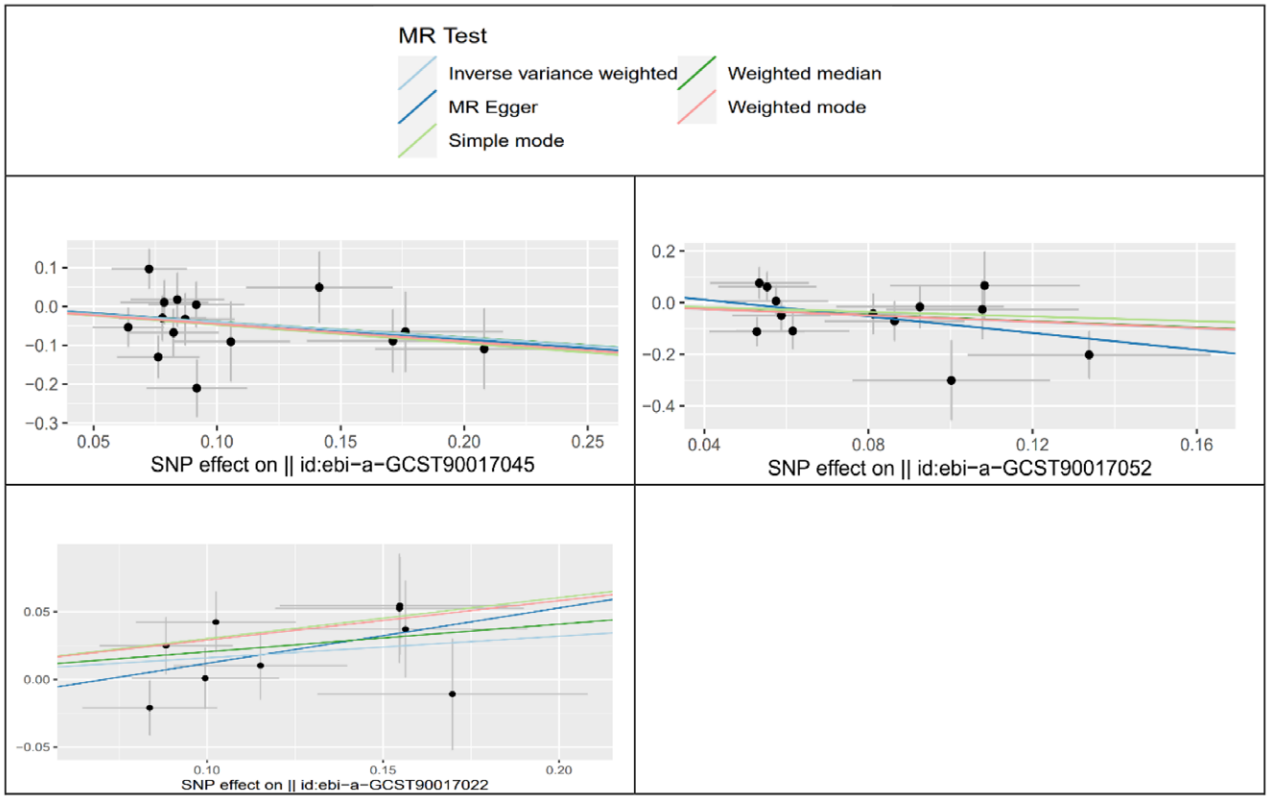

Figure 2. Scatter plots of the causal effects of the significant microbial taxa on male infertility, abnormal spermatoz and erectile dysfunction.

intake and gut microbiota, such as actinomycetes, firmicutes, Proteobacteria and Bacteroides.[24] Spermine producing gut bacteria can protect the testes from toxic exposure,[25] improve seminiferous tubules, germ cell counts, and promote the expression of genes involved in spermatogenesis. If spermatozoa are deficient, testicular dysfunction can be improved by supplementation with spermatozoa or spermatozoa-producing gut microbes.

Intestinal dysbiosis increases the number of pro-inflammatory bacteria, causes intestinal mucosal injury, high intestinal permeability and intestinal leakage, causes a large number of bacterial metabolites to enter the blood circulation, changes serum cytokine levels, leads to excessive immune response and chronic inflammatory state, and induces endothelial damage and blood-testis barrier destruction.[26] It may affect the hormone production of the testis and disturb the production and motility of sperm.[27] Bacterial translocation affects male reproductive capacity.[28] On the other hand, when the flora is disordered, the pathogenic bacteria will release endotoxin into the blood after autolysis,.[29] The increase of endotoxin level leads to male infertility, which has attracted wide attention.[30] It has been fully confirmed that endotoxemia has a causal relationship with decreased sperm motility and spermatogenesis disorders.[31] DING et al[14] found that the increase in the abundance of Prevotella in the phylum Bacteroidetes of the intestinal flora was positively correlated with the increase in circulating endotoxin, which was a potential pathogenic bacteria. Endotoxemia and epididymal inflammation are the final causes of down-regulation of testicular related genes.[32] The "leaky-gut" hypothesis also links endotoxin to hypogonadism, in which endotoxin can inhibit steroid hormone synthesis in Leydig cells and reduce pituitary luteinizing hormone drive, resulting in decreased testosterone production and ultimately reduced spermiogenesis.[33]

There is also a significant correlation between gut microbiota and behavioral emotions.[34] However, Pan et al[35] found that there may be a correlation between glutamic acid decarboxylase and semen quality in male infertility patients. Therefore, intestinal flora imbalance may also affect the emotional processing center located in the hypothalamus through neurotransmitters,[36] leading to anxiety and depression, increasing plasma and semen oxidative parameters, causing oxidative damage and damaging sperm quality.[37] In addition, neurotransmitters produced by an imbalance in the gut flora stimulate the hypothalamic-pituitary-gonadal axis, which inhibits the secretion of gonadotropin-releasing hormones Luteinizing Hormone and Follicle-stimulating hormone, resulting in a reduction in testosterone secretion. In addition, experiments in mice have confirmed that the intake of beneficial flora can change the emotional behavior of the body and the expression of central γ-aminobutyric acid receptor through the vagus nerve,[38,39] and affect hypothalamic-pituitary-gonadal axis. Alterations in the gut microbiota may also lead to increased serum trimethylamino oxide levels and promote vascular inflammation, which in turn leads to cavernous endothelial and smooth muscle cell damage and ultimately to the progression of erectile dysfunction.[40]

Intracellular flora imbalance can lead to male infertility due to trace element deficiency, abnormal metabolic regulation, and intestinal inflammation due to endotoxemia. It can also affect male fertility by affecting the host's cognitive mood or by modulating reproductive function through neural networks. The combination of the above factors is the internal mechanism of male infertility.

Ruminiclostridm6 is closely related to a variety of digestive disorders. It is important in digestion, absorption and metabolism, and can be involved in the synthesis of secreted polysaccharides. In this study, Ruminiclostridm6 was found to be significantly and negatively associated with male infertility. If the numbers of these bacteria increase, they can induce dendritic cells to secrete inflammatory factors. The activated macrophages and dendritic cells infiltrating the epididymal cavity may capture normal sperm and induce immune damage,[41] affecting

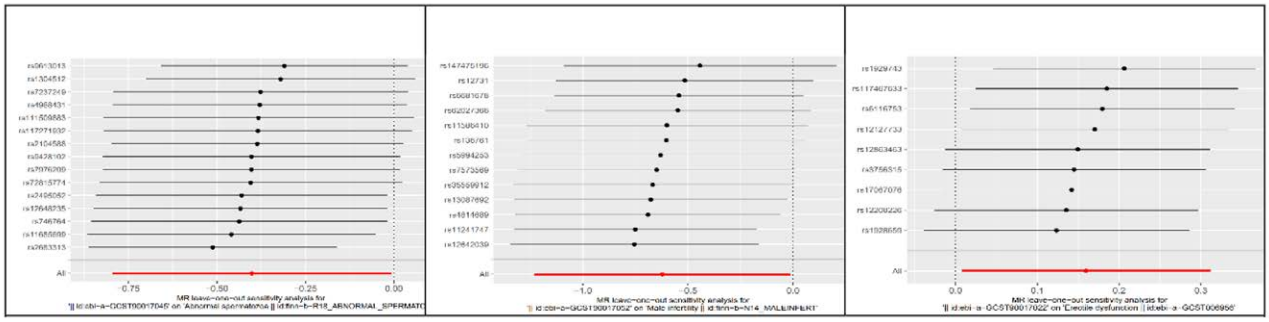

Figure 3. Leave-one-out analysis of the causal effects of the significant microbial taxa on male infertility, abnormal spermatoz and erectile dysfunction.

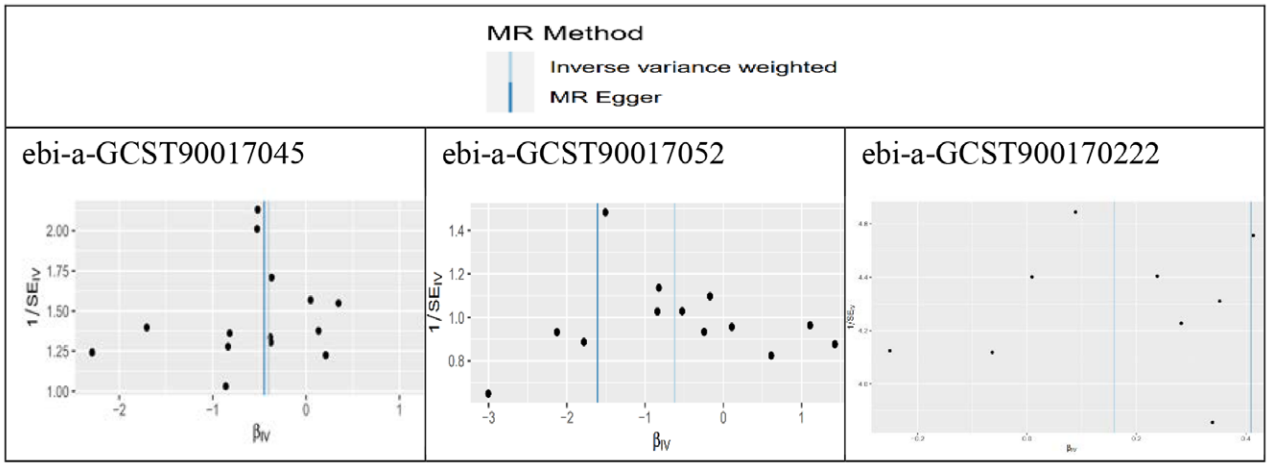

Figure 4. Funnel plots of the causal effects of the significant microbial taxa on male infertility, abnormal spermatoz and erectile dysfunction.

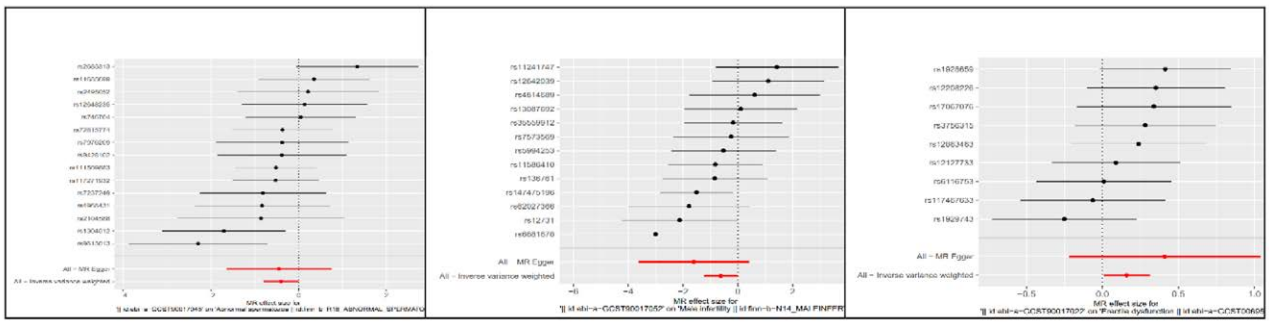

Figure 5. Forest plots of the causal effects of the significant microbial taxa on male infertility, abnormal spermatoz and erectile dysfunction.

the male reproductive function. Prevotella9 is strongly associated with abnormalities in male sperm. Ning Ding et al found that the abundance of Bacteroides and Prevotella9 was negatively correlated with sperm motility through the fecal microbiota transfer technique.[14] Transplantation of fecal microbiota from high-fat diet mice into normal diet mice led to a significant increase in Bacteroides and Prevotella in the gut,[14] resulting in local inflammation and endotoxemia, interfering with sperm epigenetics affecting spermatogenesis, and causing metabolic disorders.[14,42] This is consistent with the results of the present study. Obesity and metabolic disorders affect the gut microbial ecosystem. This is due to a significant increase in Bacteroides, Prevotella, Rickettsia, and Lactobacillus in the gut microbiota caused by a high-fat diet, resulting in a concomitant increase in endotoxin levels in the circulation. Endotoxin significantly increases inflammatory factors and monocyte chemotactic factors in the epididymis and then inhibits the expression of testicular meiosis-related genes, resulting in a decrease in sperm count. This suggests that the gut microbiota of men who eat a high-fat diet may affect fertility. Prevotella9 helps break down proteins and carbohydrates. A low-fat, high-fiber diet has been suggested to improve glucose metabolism and insulin resistance in male infertile patients, and further improve male sperm abnormalities. These studies have revealed that gut microbiota imbalance may affect the mood of male infertility patients and worsen their fertility decline. The Lachnospiraceae NC2004 group was found to be potentially beneficial and its reduction was associated with diabetes. The bacterium is closely associated with erectile function in men. The underlying mechanism may be that the gut flora, with the help of the nervous system, regulates changes in metabolic signal transduction in the body. After insulin resistance occurs, vascular nitric oxide synthesis

disorder and insulin-induced vasodilation dysfunction occur one after another, both of which may be the pathogenic factors of male erectile dysfunction.[43] In addition, nitric oxide, as an important substance to regulate penile erection and arose brain sexual desire, its content will also be affected when intestinal flora is dysregulated, and then affect penile erection.[44] Studies and evidence on this bacterium are insufficient, and in the future probiotics could be developed for the treatment and prevention of male sexual dysfunction. Ma et al found that the changes of NK4A136 group in Trichospirillaceae were significantly correlated with the enhancement of intestinal barrier function induced by spermidine.[45] It is speculated that Lachnospiraceae may be related to the damage of amino acid metabolism.

This study is the first to investigate the causal relationship between male fertility and gut microbiota using MR methods, and various sensitivity analyses were performed to assess the robustness of the analysis. As suggested by the above results, abnormal gut microbiota can emerge to affect male fertility.

5. Limitation

A limitation of this study is that the target population exclusively comprises European populations, which may restrict the generalizability of the findings. Additionally, the study did not account for the mediating role of metabolic substances, thereby potentially limiting the robustness of the conclusions. Currently, most studies investigating the role of gut microbiota in male infertility are predominantly based on animal experiments, with a notable lack of large-scale randomized controlled trials and clinical evidence. This gap hinders the establishment of a solid clinical foundation for further exploring the mechanisms of gut microbiota in male infertility interventions and identifying effective probiotics for treatment.

6. Future directions

Animal studies have also shown that fecal microbiota transplantation can treat infertility and improve sperm quality, suggesting another link between gut dysbiosis and fertility. The improved gut microbiota by alginate-oligosaccharide can be used to promote type 2 diabetes-reduced semen quality and male subfertility associated with metabolic diseases.[46,47] Transplantation of pre-diabetic sheep feces into mice revealed elevated sphingosine levels and impaired spermatogenesis. As a potential prebiotic agent, melatonin can be used to treat male infertility by improving blood glucose and insulin resistance levels.[48] Another study in mice by Zhang et al[8] also found that fecal microbiota transplantation improved spermatogenesis, increased sperm concentration, improved motility, increased levels of testicular antioxidants and key reproductive proteins, and increased expression of genes related to spermatogenesis.[49]

Prebiotics and probiotics can, to a certain extent, increase the proportion of germ cells, promote the expression of spermatogenic genes, improve transcription factors in vivo, and improve the testicular microenvironment, thereby increasing sperm concentration and sperm motility and improving sperm quality.[8,50] The results of the current study provide new insights into the mechanisms of action of beneficial gut microbiota in treating men with high risk of infertility who should be treated individually and in combination with their own gut microbiota composition. To find the intestinal microbiota associated with male infertility, with the aim of identifying transgenic probiotics, it is necessary to explore microbiota therapy to prevent and treat current fertility problems by regulating body homeostasis and protecting intestinal mucosal barrier.

7. Conclusion

In this study, the causal relationship between Ruminiclostridm6, Prevotella9, Lachnospiraceae NC2004 group and male fertility was demonstrated by MR analysis, which provides a theoretical basis to guide clinical work and can point the way for future research. The hope is that underlying male fertility problems can be treated with probiotics.

Acknowledgments

We wish to acknowledge the participants and investigators of the GWAS used in current study.

Author contributions

Conceptualization: Zhi-hong Wang, Yi-fan Kang.
Data curation: Zhi-hong Wang, Yi-fan Kang.
Formal analysis: Zhi-hong Wang, Yi-fan Kang.
Funding acquisition: Zhi-hong Wang.
Investigation: Zhi-hong Wang, Yi-fan Kang.
Methodology: Zhi-hong Wang, Yi-fan Kang.
Project administration: Zhi-hong Wang, Yi-fan Kang.
Resources: Zhi-hong Wang, Yi-fan Kang.
Software: Yi-fan Kang.
Supervision: Zhi-hong Wang, Yi-fan Kang.
Validation: Yi-fan Kang.
Visualization: Zhi-hong Wang, Yi-fan Kang.
Writing – original draft: Zhi-hong Wang, Yi-fan Kang.
Writing – review & editing: Zhi-hong Wang.

References

[1] Agarwal A, Baskaran S, Parekh N, et al. Male infertility. Lancet. 2021;397:319–33.
[2] Ferlin A, Arredi B, Speltra E, et al. Molecular and clinical characterization of Y chromosome microdeletions in infertile men: a 10-year experience in Italy. J Clin Endocrinol Metab. 2007;92:762–70.
[3] Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. Science. 2005;308:1635–8.
[4] Magne F, Gotteland M, Gauthier L, et al. The firmicutes/bacteroidetes ratio: a relevant marker of gut dysbiosis in obese patients? Nutrients. 2020;12:1474.
[5] Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. Microb Ecol Health Dis. 2015;26:26191.
[6] Proal AD, Lindseth IA, Marshall TG. Microbe-microbe and host-microbe interactions drive microbiome dysbiosis and inflammatory processes. Discov Med. 2017;23:51–60.
[7] Allegretti JR, Mullish BH, Kelly C, Fischer M. The evolution of the use of faecal microbiota transplantation and emerging therapeutic indications. Lancet. 2019;394:420–31.
[8] Zhang P, Feng Y, Li L, et al. Improvement in sperm quality and spermatogenesis following faecal microbiota transplantation from alginate oligosaccharide dosed mice. Gut. 2021;70:222–5.
[9] Zhang C, Xiong B, Chen L, et al. Rescue of male fertility following faecal microbiota transplantation from alginate oligosaccharide-dosed mice. Gut. 2021;70:2213–5.
[10] Liang Y, Zeng W, Hou T, et al. Gut microbiome and reproductive endocrine diseases: a Mendelian randomization study. Front Endocrinol (Lausanne). 2023;14:1164186.
[11] Fox N, Mathers N. Empowering research: statistical power in general practice research. Fam Pract. 1997;14:324–9.
[12] Ayad B, Omolaoye TS, Louw N, et al. Oxidative stress and male infertility: evidence from a research perspective. Front Reprod Health. 2022;4:822257.
[13] Grüner N, Ortlepp AL, Mattner J. Pivotal role of intestinal microbiota and intraluminal metabolites for the maintenance of gut-bone physiology. Int J Mol Sci . 2023;24:5161.
[14] Ding N, Zhang X, Zhang XD, et al. Impairment of spermatogenesis and sperm motility by the high-fat diet-induced dysbiosis of gut microbes. Gut. 2020;69:1608–19.
[15] Gorman A, Golovanov AP. Lipopolysaccharide structure and the phenomenon of low endotoxin recovery. Eur J Pharm Biopharm. 2022;180:289–307.
[16] Zhang F-L, Yuan S, Dong P-Y, et al. Multi-omics analysis reveals that iron deficiency impairs spermatogenesis by gut-hormone synthesis axis. Ecotoxicol Environ Saf. 2022;248:114344.

[17] Cook SP, Brokaw CJ, Muller CH, Babcock DF. Sperm chemotaxis: egg peptides control cytosolic calcium to regulate flagellar responses. Dev Biol. 1994;165:10–9.
[18] Hadwan MH, Almashhedy LA, Alsalman AR. Oral zinc supplementation restores high molecular weight seminal zinc binding protein to normal value in Iraqi infertile men. BMC Urol. 2012;12:32.
[19] Zhang T, Sun P, Geng Q, et al. Disrupted spermatogenesis in a metabolic syndrome model: the role of vitamin A metabolism in the gut-testis axis. Gut. 2022;71:78–87.
[20] Yuan C, Xiang L, Jian Z, Liao B. Vitamin D levels and risk of male factor infertility: a mendelian randomization study. World J Mens Health. 2023;41:640–8.
[21] Yamaguchi Y, Adachi K, Sugiyama T, et al. Association of intestinal microbiota with metabolic markers and dietary habits in patients with type 2 diabetes. Digestion. 2016;94:66–72.
[22] Ma J, Han RY, Mei XA, et al. Correlation analysis of insulin resistance index with male reproductive hormone levels and semen parameters. Zhonghua Nan Ke Xue. 2018;24:5.
[23] Pegg AE. The function of spermine. IUBMB Life. 2014;66:8–18.
[24] Pegg AE, Michael AJ. Spermine synthase. Cell Mol Life Sci. 2010;67:113–21.
[25] Zhao Q, Huang JF, Cheng Y, et al. Polyamine metabolism links gut microbiota and testicular dysfunction. Microbiome. 2021;9:224.
[26] Al-Asmakh M, Stukenborg JB, Reda A, et al. The gut microbiota and developmental programming of the testis in mice. PLoS One. 2014;9:e103809.
[27] Shen P, Ji S, Li X, et al. LPS-induced systemic inflammation caused mPOA-FSH/LH disturbance and impaired testicular function. Front Endocrinol (Lausanne). 2022;13:886085.
[28] Venneri MA, Franceschini E, Sciarra F, Rosato E, D'Ettorre G, Lenzi A. Human genital tracts microbiota: dysbiosis crucial for infertility. J Endocrinol Invest. 2022;45:1151–60.
[29] Wang N, Chen L, Yi K, Zhang B, Li C, Zhou X. The effects of microbiota on reproductive health: a review. Crit Rev Food Sci Nutr. 2024;64:1486–507.
[30] Wen JW. Intestinal microbiota disorder and male infertility: an important cause that is easily overlooked. Chin Med J. 2020;100:3.
[31] Cao T, Wang S, Pan Y, et al. Characterization of the semen, gut, and urine microbiota in patients with different semen abnormalities. Front Microbiol. 2023;14:1182320.
[32] Alvarez JG, Sharma RK, Ollero M, et al. Increased DNA damage in sperm from leukocytospermic semen samples as determined by the sperm chromatin structure assay. Fertil Steril. 2002;78:319–29.
[33] Tremellen K. Gut Endotoxin Leading to a Decline IN Gonadal function (GELDING) - a novel theory for the development of late onset hypogonadism in obese men. Basic Clin Androl. 2016;26:7.
[34] Jiang HY, Zhang X, Yu ZH, et al. Altered gut microbiota profile in patients with generalized anxiety disorder. J Psychiatr Res. 2018;104:130–6.
[35] Pan Y, Wang S, Kang J, et al. Association between generalized anxiety symptoms and semen quality in infertile men: a multicentre study in North China. Andrologia. 2022;54:e14449.
[36] Öztekin U, Hacimusalar Y, Gurel A, Karaaslan O. The relationship of male infertility with somatosensory amplification, health anxiety and depression levels. Psychiatry Investig. 2020;17:350–5.
[37] Gumus K, Gulum M, Yeni E, et al. Effects of psychological status on the oxidation parameters of semen and blood in azoospermic men. Urol J. 2019;16:295–9.
[38] Mohajeri MH, La Fata G, Steinert RE, Weber P. Relationship between the gut microbiome and brain function. Nutr Rev. 2018;76:481–96.
[39] Fung TC, Olson CA, Hsiao EY. Interactions between the microbiota, immune and nervous systems in health and disease. Nat Neurosci. 2017;20:145–55.
[40] Gabrielsen JS, Tanrikut C. Chronic exposures and male fertility: the impacts of environment, diet, and drug use on spermatogenesis. Andrology. 2016;4:648–61.
[41] Zheng W, Zhang S, Chen X, Jiang S, Li Z, Li M. Case report: dendritic cells and macrophages capture sperm in chronically inflamed human epididymis. Front Immunol. 2021;12:629680.
[42] Belloc S, Cohen-Bacrie M, Amar E, et al. High body mass index has a deleterious effect on semen parameters except morphology: results from a large cohort study. Fertil Steril. 2014;102:1268–73.
[43] Kara E, Kahraman E, Dayar E, et al. The role of resistin on metabolic syndrome-induced erectile dysfunction and the possible therapeutic effect of Boldine. Andrology. 2020;8:1728–35.
[44] Dutta S, Sengupta P. The role of nitric oxide on male and female reproduction. Malays J Med Sci. 2022;29:18–30.
[45] Ma L, Ni Y, Wang Z, et al. Spermidine improves gut barrier integrity and gut microbiota function in diet-induced obese mice. Gut Microbes. 2020;12:1–19.
[46] Yan X, Feng Y, Hao Y, et al. Gut-testis axis: microbiota prime metabolome to increase sperm quality in young type 2 diabetes. Microbiol Spectr. 2022;10:e0142322.
[47] Hao Y, Feng Y, Yan X, et al. Gut microbiota-testis axis: FMT mitigates high-fat diet-diminished male fertility via improving systemic and testicular metabolome. Microbiol Spectr. 2022;10:e0002822.
[48] Sun Y, Sun P, Hu Y, et al. Elevated testicular apoptosis is associated with elevated sphingosine driven by gut microbiota in prediabetic sheep. BMC Biol. 2022;20:121.
[49] Li H, Li N, Lu Q, et al. Chronic alcohol-induced dysbiosis of the gut microbiota and gut metabolites impairs sperm quality in mice. Front Microbiol. 2022;13:1042923.
[50] Zhao Y, Zhang P, Ge W, et al. Alginate oligosaccharides improve germ cell development and testicular microenvironment to rescue busulfan disrupted spermatogenesis. Theranostics. 2020;10:3308–24.