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# Causal Effects of Gut Microbiota on Abnormal Spermatozoa: A Two-Sample Mendelian Randomization Study

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**Abstract:** Several clinical studies have reported the link between gut microbiota and abnormal spermatozoa. However, whether gut microbiota plays a causal role in abnormal spermatozoa remains unclear. The present study used genome-wide association study (GWAS) summary data to perform two-sample Mendelian randomization to investigate causal associations between the gut microbiota and abnormal spermatozoa. SNPs that showed significant correlations with genus at genome-wide significance thresholds (P < 1× 10-5, F >10) were identified as potential instrumental variables. The inverse variance weighted method was used as the primary analysis. The results showed a causal effect of gut microbiota components on abnormal spermatozoa. Erysipelotrichaceae, Streptococcaceae, Coprococcus1, RuminococcaceaeUCG009, Streptococcus, and Erysipelotrichales were positively correlated with the risk of abnormal spermatozoa. And Porphyromonadaceae and Prevotella9 were negatively correlated with abnormal spermatozoa. Our analyses prove a potential causal relationship between several gut microbiota taxa and abnormal spermatozoa. This study supports that there are beneficial or detrimental causal effects of gut microbiota components on abnormal spermatozoa. However, more studies are necessary to reveal the impact of gut microbiota on abnormal spermatozoa.

**Keywords:** Gut Microbiota; Abnormal Spermatozoa; Mendelian Randomization; Single Nucleotide Polymorphism.

## 1. Introduction

Infertility affects 8-12% of couples worldwide, with the male factor being an important cause or contributing factor[1]. It's reported that 20-

30% of infertility cases are caused by the male alone, and another 20% involve a contributing male cause[2]. A variety of factors and risk factors are involved in the rising incidence of male infertility[3], which can be classified as congenital, acquired, and idiopathic[1, 3]. Approximately 30-50% of male infertility cases are idiopathic, with no identifiable cause[4]. Therefore, the causes of male infertility are varied and, in most cases, poorly understood[5]. Infertility leads to significant psychological and social distress. It is also a severe economic burden for patients and healthcare systems[1, 6]. More seriously, male infertility contributes to a higher incidence of cancer[7]. Fertilization in mammals depends on the stability of many biological processes, and sperm quality is essential for the penetration and activation of the sperm in the oocyte[8]. Abnormal spermatozoa may lead to a decrease in achieving a pregnancy[9]. Approximately 2% of infertile men report abnormal sperm parameters[10].

The gut microbiota is essential in helping the body absorb nutrients from food[11]. It has a significant impact on our health, and many diseases are associated with imbalances in microbial composition or ecological disturbances, such as IBD, rheumatoid diseases, atopic diseases, cancer, metabolic syndrome, and even psychiatric disorders[12]. Gut microbiota may play a role in male reproductive health[13]. Studies have shown that chronic alcohol exposure damages the gut microbiota of mice, which in turn leads to metabolic disorders, elevated serum endotoxin and inflammatory cytokine levels, testicular inflammation, abnormalities in the expression of relevant genes, and ultimately impaired spermatozoa in mice[14], another study has shown that treatment of the rat gut microbiota improved spermatogenic function in rats[15]. Significant differences existed between the gut microbiota of fertile and infertile samples in Actinobacteria, Bacteroidetes, and Firmicutes[16]. Gut microbiota translocation not only leads to testicular and epididymal inflammation by activating the immune system but also induces insulin resistance along with gastrointestinal hormones such as leptin and growth hormone-releasing peptide, which in turn affects the secretion of various sex hormones, such as LH, FSH, and T regulating spermatogenesis. In addition, by controlling and metabolizing androgens and affecting the blood-testis barrier, the gut microbiota can influence spermatogenesis.[13]

Mendelian randomization（MR）is a method that uses genetic variants to determine whether an observational association between a risk factor and an outcome is consistent with a causal effect[13]. Due to the limitations of observational studies, which cannot avoid the influence of confounding factors, causality has yet to be established for many risk factors associated with disease[18]. This makes MR a powerful method for addressing questions in human biology and epidemiology, including the relationship between gut microbiota and disease[18]. In this study, we explore the relationship between gut microbiota and abnormal spermatozoa by utilizing Mendelian randomization to provide new guidance for managing patients with abnormal spermatozoa.

## 2. Materials and Methods

### 2.1. Study Design

This study aims to analyze the causal relationship between gut microbiota and the risk of abnormal spermatozoa. The

genetic variants used in two-sample Mendelian randomization analysis must: a) be strongly associated with gut microbiota, b) be not associated with any confounder of gut microbiota and abnormal spermatozoa, c) be not related to the relevant outcomes obtained through other methods[19].

## 2.2. Data Sources

The human gut microbiota summary statistics used in this study come from the GWAS meta-analysis, including 18,340 participants from 24 cohorts[20]. Association analyses were performed adjusting for age, sex, technical covariates, and genetic principal components using coordinated 16S rRNA gene sequencing profiles and genotyping data from cohorts in the United States, Canada, Germany, Denmark, the Netherlands, Belgium, Sweden, Finland, and the United Kingdom[20]. A total of 196 taxa were included in the analysis.

## 2.3. Data Outcome

Outcome data was searched from the Ieu Open GWAS Project database (https://gwas.mrcieu.ac.uk/). In our research, the Phenotype "abnormal spermatozoa" was adopted, and this data included 915 cases and 209,006 controls. We selected participants of European origin for the cohort to avoid bias due to the stratification effects of factors such as ancestry and population. Herein, we extracted the overall Abnormal spermatozoa beta coefficients and standard errors for each retrieved SNP of gut microbiota summary statistics.

## 2.4. Instrumental Variable Filtering

In the first step, Single Nucleotide Polymorphisms (SNP) that showed significant correlations with genus at genome-wide significance thresholds ($P < 1\times 10\text{-}5$, $F > 10$) were identified as potential instrumental variables (IVs). To ensure the independence of the selected SNPs, a linkage imbalance threshold ($R2 < 0.001$) and a linkage disequilibrium threshold of 10,000 kb were applied. Unclear, duplicated, or palindromic single nucleotide polymorphisms duplicated or palindromic were removed to ensure consistent SNP alignment in the assay and results. MR-Egger intercept was sued to analysis for the horizontal pleiotropy. At last, we used Leave-one-out analysis to assess if each IV affected the overall estimates of the rest of the IVs, and the heterogeneity tests were used to identify if they were influenced by heterogeneity bias[18].

## 2.5. MR Analysis

Five methods of 2SMR were used to estimate the causal association between gut microbiota and the risk of abnormal spermatozoa, including MR Egger, Weighted median, Inverse variance weighted, Simple mode, and Weighted mode. We consider IVW the primary analysis method because it provides the most accurate effect estimates, and almost all MR analyses use it as the preliminary analysis[21].

# 3. Results

## 3.1. Selected SNPs and IVs Validation

We obtained 116 SNPs associated with three families, four genus, and one order at the significant difference level (p-value $< 1\times 10\text{-}5$), and they all surpassed the limited value ((clumping_distance = 10,000kb and clumping_r2 < 0.001) in linkage disequilibrium analysis; what's more, they with F-statistics > 10. (Table 1).

**Table 1.** SNPs used as instrumental variables of the gut microbiota

| Bacterial taxa | SNP | EA | OA | Beta | Se | Pvalue | Sample size | F |
|---|---|---|---|---|---|---|---|---|
| Erysipelotrichaceae | rs7234058 | T | C | -0.0946 | 0.0194 | 9.12E-07 | 16874 | 23.7410 |
| | rs62504403 | C | T | 0.0681 | 0.0128 | 1.12E-07 | 16981 | 28.3673 |
| | rs17530232 | A | G | 0.1030 | 0.0225 | 2.79E-06 | 16622 | 21.0397 |
| | rs2300774 | G | A | 0.0524 | 0.0107 | 8.95E-07 | 18216 | 24.0911 |
| | rs35161940 | T | C | -0.0806 | 0.0168 | 1.85E-06 | 18213 | 23.1157 |
| | rs10781552 | C | T | -0.0552 | 0.0116 | 2.33E-06 | 17789 | 22.6304 |
| | rs8003149 | C | T | 0.0539 | 0.0117 | 4.08E-06 | 18213 | 21.2454 |
| | rs4078432 | C | T | -0.0609 | 0.0134 | 4.23E-06 | 17789 | 20.7209 |
| | rs1074800 | A | G | 0.0492 | 0.0109 | 6.15E-06 | 17789 | 20.4567 |
| | rs56970041 | T | G | 0.0724 | 0.0164 | 5.40E-06 | 18217 | 19.3832 |
| | rs7826267 | T | G | -0.0839 | 0.0199 | 9.28E-06 | 17586 | 17.7530 |
| | rs1884466 | C | T | -0.0475 | 0.0107 | 9.53E-06 | 18205 | 19.7576 |
| | rs290833 | T | G | -0.0497 | 0.0111 | 8.03E-06 | 17789 | 19.9402 |
| Porphyromonadaceae | rs10119172 | C | G | -0.0737 | 0.0143 | 1.32E-07 | 18022 | 26.7339 |
| | rs10858364 | G | T | 0.0553 | 0.0121 | 4.31E-06 | 18028 | 20.9488 |
| | rs17065783 | A | G | -0.0591 | 0.0122 | 1.79E-06 | 18028 | 23.3960 |
| | rs6953849 | A | G | 0.0718 | 0.0151 | 2.44E-06 | 18028 | 22.7008 |
| | rs3111851 | C | G | 0.0582 | 0.0121 | 2.19E-06 | 18024 | 23.0676 |
| | rs7330827 | T | C | -0.1038 | 0.0237 | 8.05E-06 | 13754 | 19.1360 |
| | rs35961441 | A | C | 0.0915 | 0.0207 | 8.37E-06 | 16374 | 19.4893 |
| | rs10762312 | G | A | -0.0524 | 0.0119 | 8.70E-06 | 18028 | 19.4272 |

| Bacterial taxa | SNP | EA | OA | Beta | Se | Pvalue | Sample size | F |
|---|---|---|---|---|---|---|---|---|
| | rs35233670 | T | C | -0.0474 | 0.0106 | 7.91E-06 | 18024 | 19.9526 |
| | rs864093 | A | C | -0.0528 | 0.0117 | 9.60E-06 | 18027 | 20.1827 |
| | rs1980561 | A | G | -0.0485 | 0.0109 | 8.95E-06 | 18028 | 19.7193 |
| Streptococcaceae | rs2952251 | A | G | -0.0639 | 0.0127 | 3.72E-07 | 16612 | 25.5272 |
| | rs4968759 | A | G | -0.0544 | 0.0111 | 8.92E-07 | 16624 | 23.9021 |
| | rs395407 | G | C | -0.0827 | 0.0173 | 1.33E-06 | 16088 | 22.9639 |
| | rs11110281 | T | C | -0.1306 | 0.0226 | 1.40E-08 | 15316 | 33.3831 |
| | rs6563952 | G | C | 0.0802 | 0.0179 | 8.71E-06 | 16627 | 20.1640 |
| | rs35344081 | G | A | 0.0609 | 0.0130 | 2.64E-06 | 16624 | 22.0688 |
| | rs2370083 | G | T | -0.0843 | 0.0185 | 4.26E-06 | 16516 | 20.8597 |
| | rs76717940 | T | A | 0.1506 | 0.0334 | 3.09E-06 | 7720 | 20.3175 |
| | rs10028567 | C | T | -0.0934 | 0.0190 | 3.72E-06 | 16549 | 24.0763 |
| | rs16950051 | A | G | 0.1070 | 0.0237 | 5.34E-06 | 14431 | 20.3878 |
| | rs77968078 | G | A | -0.0993 | 0.0225 | 7.93E-06 | 15519 | 19.5123 |
| | rs7916711 | A | G | 0.0960 | 0.0215 | 6.33E-06 | 15515 | 19.8365 |
| | rs957755 | T | G | -0.0642 | 0.0143 | 7.42E-06 | 16627 | 20.2658 |
| | rs6806351 | T | C | -0.0619 | 0.0136 | 6.94E-06 | 16198 | 20.8056 |
| | rs9903102 | C | A | -0.0693 | 0.0154 | 4.92E-06 | 16623 | 20.2232 |
| | rs57646748 | G | A | -0.0880 | 0.0199 | 7.88E-06 | 16160 | 19.6093 |
| Coprococcus1 | rs56405618 | A | G | -0.0896 | 0.0187 | 1.57E-06 | 16379 | 23.0918 |
| | rs4277593 | G | A | -0.0586 | 0.0110 | 1.14E-07 | 16651 | 28.3870 |
| | rs1519491 | T | C | 0.0499 | 0.0114 | 8.95E-06 | 16733 | 19.3223 |
| | rs74101919 | T | C | -0.0719 | 0.0145 | 1.03E-06 | 16732 | 24.7029 |
| | rs1010560 | C | A | 0.0580 | 0.0123 | 1.96E-06 | 16723 | 22.3517 |
| | rs73031725 | T | C | 0.1676 | 0.0355 | 1.98E-06 | 7206 | 22.2524 |
| | rs1576241 | A | G | -0.0510 | 0.0110 | 3.33E-06 | 16729 | 21.7056 |
| | rs73167075 | T | C | 0.0573 | 0.0128 | 8.57E-06 | 16733 | 20.1812 |
| | rs12794898 | G | T | 0.0903 | 0.0197 | 4.92E-06 | 15186 | 20.9767 |
| | rs946513 | C | T | 0.2059 | 0.0460 | 8.62E-06 | 4310 | 20.0243 |
| | rs1762123 | C | T | -0.0892 | 0.0199 | 8.01E-06 | 16724 | 20.1588 |
| | rs7784490 | C | G | -0.0519 | 0.0113 | 4.55E-06 | 16733 | 21.0047 |
| | rs12886051 | G | C | -0.0522 | 0.0118 | 8.01E-06 | 16633 | 19.5339 |
| | rs2907920 | A | G | 0.0561 | 0.0127 | 7.65E-06 | 16040 | 19.5766 |
| Prevotella9 | rs111509883 | T | C | 0.1711 | 0.0348 | 1.24E-06 | 6960 | 24.2276 |
| | rs2683313 | A | G | -0.0725 | 0.0152 | 1.69E-06 | 10260 | 22.8386 |
| | rs117271932 | A | G | 0.2081 | 0.0440 | 2.82E-06 | 4878 | 22.3171 |
| | rs10512344 | C | G | 0.2474 | 0.0544 | 3.19E-06 | 3063 | 20.6578 |
| | rs9428102 | A | G | -0.0779 | 0.0176 | 4.62E-06 | 10264 | 19.5639 |
| | rs746764 | T | C | -0.0916 | 0.0193 | 2.04E-06 | 9385 | 22.4526 |
| | rs16966465 | G | C | 0.0743 | 0.0165 | 9.33E-06 | 10261 | 20.2210 |
| | rs2104588 | T | C | 0.1056 | 0.0238 | 8.13E-06 | 9268 | 19.7117 |
| | rs11199734 | A | T | 0.0770 | 0.0169 | 0.000007 | 10263 | 20.6532 |
| | rs7232121 | G | C | 0.0671 | 0.0144 | 3.76E-06 | 9790 | 21.6583 |
| | rs11685699 | C | T | -0.1414 | 0.0296 | 2.03E-06 | 8342 | 22.8525 |
| | rs9613013 | G | A | 0.0918 | 0.0203 | 6.1E-06 | 9790 | 20.4888 |
| | rs2495052 | A | G | 0.0839 | 0.0189 | 8.97E-06 | 9790 | 19.8008 |
| | rs72815774 | T | C | -0.1762 | 0.0393 | 8.78E-06 | 5728 | 20.0842 |

| Bacterial taxa | SNP | EA | OA | Beta | Se | Pvalue | Sample size | F |
|---|---|---|---|---|---|---|---|---|
| | rs4968431 | G | T | 0.0640 | 0.0144 | 8.58E-06 | 10271 | 19.7118 |
| | rs7976209 | T | C | -0.0871 | 0.0198 | 7.28E-06 | 10263 | 19.3475 |
| | rs12648235 | T | C | 0.0786 | 0.0178 | 7.39E-06 | 10271 | 19.5613 |
| | rs1304512 | G | A | 0.0762 | 0.0166 | 5.29E-06 | 9790 | 21.0739 |
| | rs7237249 | C | T | -0.0824 | 0.0182 | 8.93E-06 | 10271 | 20.4540 |
| Ruminococcaceae UCG009 | rs8009993 | G | C | -0.1359 | 0.0245 | 4.42E-08 | 7389 | 30.8126 |
| | rs1550196 | G | A | 0.1308 | 0.0262 | 1.13E-06 | 7389 | 24.8421 |
| | rs4708333 | T | G | -0.0840 | 0.0175 | 1.56E-06 | 7511 | 23.1161 |
| | rs61779334 | G | C | -0.1381 | 0.0292 | 1.94E-06 | 6865 | 22.3622 |
| | rs4079028 | C | T | 0.0916 | 0.0199 | 3.28E-06 | 7480 | 21.0882 |
| | rs2058609 | A | G | 0.0816 | 0.0175 | 3.12E-06 | 7510 | 21.8311 |
| | rs12508214 | C | T | -0.0775 | 0.0169 | 4.75E-06 | 7511 | 21.0206 |
| | rs9558661 | T | C | -0.0898 | 0.0201 | 7.01E-06 | 7480 | 19.9988 |
| | rs758191 | T | G | 0.1770 | 0.0375 | 9.01E-06 | 5829 | 22.2622 |
| | rs2192926 | A | G | -0.0890 | 0.0193 | 4.88E-06 | 7511 | 21.2847 |
| | rs113006825 | T | C | -0.0929 | 0.0208 | 7.98E-06 | 7165 | 19.9314 |
| | rs138460696 | A | G | 0.1393 | 0.0316 | 9.81E-06 | 6865 | 19.4449 |
| | rs6952765 | G | A | 0.0732 | 0.0167 | 8.13E-06 | 7511 | 19.2643 |
| | rs78410648 | A | G | 0.1210 | 0.0277 | 9.67E-06 | 7510 | 19.0293 |
| Streptococcus | rs11110281 | T | C | -0.1375 | 0.0227 | 2.58E-09 | 15120 | 36.5673 |
| | rs4968759 | A | G | -0.0515 | 0.0112 | 3.78E-06 | 16384 | 21.1242 |
| | rs11764382 | A | G | -0.0695 | 0.0144 | 1.29E-06 | 16387 | 23.4211 |
| | rs1918540 | G | A | 0.0596 | 0.0128 | 2.44E-06 | 16373 | 21.6563 |
| | rs17708276 | A | G | -0.0794 | 0.0171 | 3.04E-06 | 16278 | 21.6491 |
| | rs10448310 | A | G | -0.0518 | 0.0111 | 3.31E-06 | 16384 | 21.6433 |
| | rs11720390 | G | A | 0.1070 | 0.0228 | 3.59E-06 | 15220 | 22.0076 |
| | rs395407 | G | C | -0.0793 | 0.0174 | 4.37E-06 | 15864 | 20.8288 |
| | rs7916711 | A | G | 0.1029 | 0.0217 | 2.72E-06 | 15294 | 22.4042 |
| | rs71481756 | T | G | 0.0931 | 0.0208 | 6.51E-06 | 14526 | 20.0433 |
| | rs2370083 | G | T | -0.0817 | 0.0186 | 9.75E-06 | 16277 | 19.3146 |
| | rs10028567 | C | T | -0.0921 | 0.0192 | 7.3E-06 | 16311 | 23.0440 |
| | rs6806351 | T | C | -0.0634 | 0.0137 | 4.94E-06 | 15973 | 21.5123 |
| | rs57646748 | G | A | -0.0908 | 0.0200 | 5.48E-06 | 15939 | 20.5245 |
| | rs9903102 | C | A | -0.0709 | 0.0155 | 4.18E-06 | 16384 | 20.8751 |
| | rs6563952 | G | C | 0.0827 | 0.0180 | 5.82E-06 | 16387 | 21.1156 |
| Erysipelotrichales | rs7234058 | T | C | -0.0946 | 0.0194 | 9.12E-07 | 16874 | 23.7410 |
| | rs17530232 | A | G | 0.1030 | 0.0225 | 2.79E-06 | 16622 | 21.0397 |
| | rs2300774 | G | A | 0.0524 | 0.0107 | 8.95E-07 | 18216 | 24.0911 |
| | rs35161940 | T | C | -0.0806 | 0.0168 | 1.85E-06 | 18213 | 23.1157 |
| | rs10781552 | C | T | -0.0552 | 0.0116 | 2.33E-06 | 17789 | 22.6304 |
| | rs8003149 | C | T | 0.0539 | 0.0117 | 4.08E-06 | 18213 | 21.2454 |
| | rs62504403 | C | T | 0.0681 | 0.0128 | 1.12E-07 | 16981 | 28.3673 |
| | rs4078432 | C | T | -0.0609 | 0.0134 | 4.23E-06 | 17789 | 20.7209 |
| | rs1074800 | A | G | 0.0492 | 0.0109 | 6.15E-06 | 17789 | 20.4567 |
| | rs56970041 | T | G | 0.0724 | 0.0164 | 5.4E-06 | 18217 | 19.3832 |
| | rs7826267 | T | G | -0.0839 | 0.0199 | 9.28E-06 | 17586 | 17.7530 |
| | rs1884466 | C | T | -0.0475 | 0.0107 | 9.53E-06 | 18205 | 19.7576 |
| | rs290833 | T | G | -0.0497 | 0.0111 | 8.03E-06 | 17789 | 19.9402 |

## 3.2. MR Results

**Table 2.** MR results of causal links between the gut microbiota and abnormal spermatozoa. (SNP, Single nucleotide polymorphism; OR, Odds ratio; IVW, Inverse variance weighted; CI, Confidence interval.)

| Bacterial Taxa (Exposure) | Nsnp | Methods | P-value | OR | 95%CI |
|---|---|---|---|---|---|
| Family Erysipelotrichaceae | 13 | IVW | 0.0384 | 1.77 | 1.03-3.03 |
| | 13 | MR Egger | 0.3025 | 3.62 | 0.35-37.31 |
| | 13 | Weighted median | 0.2724 | 1.51 | 0.72-3.14 |
| | 13 | Simple mode | 0.5815 | 1.39 | 0.45-4.30 |
| | 13 | Weighted mode | 0.5085 | 1.49 | 0.48-4.65 |
| Family Porphyromonadaceae | 11 | IVW | 0.0233 | 0.50 | 0.28-0.91 |
| | 11 | MR Egger | 0.9144 | 0.85 | 0.05-14.23 |
| | 11 | Weighted median | 0.1139 | 0.54 | 0.25-1.16 |
| | 11 | Simple mode | 0.4446 | 0.59 | 0.16-2.17 |
| | 11 | Weighted mode | 0.3901 | 0.59 | 0.18-1.87 |
| Family Streptococcaceae | 16 | IVW | 0.0013 | 2.06 | 1.33-3.21 |
| | 16 | MR Egger | 0.0370 | 6.63 | 1.33-33.13 |
| | 16 | Weighted median | 0.0029 | 2.45 | 1.36-4.42 |
| | 16 | Simple mode | 0.0350 | 3.22 | 1.20-8.64 |
| | 16 | Weighted mode | 0.0319 | 3.18 | 1.22-8.30 |
| Genus Coprococcus1 | 14 | IVW | 0.0068 | 1.87 | 1.19-2.94 |
| | 14 | MR Egger | 0.3970 | 1.67 | 0.53-5.29 |
| | 14 | Weighted median | 0.1058 | 1.70 | 0.89-3.23 |
| | 14 | Simple mode | 0.2220 | 1.93 | 0.71-5.30 |
| | 14 | Weighted mode | 0.2474 | 1.73 | 0.71-4.20 |
| Genus Prevotella9 | 19 | IVW | 0.0306 | 0.69 | 0.50-0.97 |
| | 19 | MR Egger | 0.3272 | 0.61 | 0.23-1.60 |
| | 19 | Weighted median | 0.0524 | 0.66 | 0.43-1.00 |
| | 19 | Simple mode | 0.1653 | 0.60 | 0.30-1.20 |
| | 19 | Weighted mode | 0.1744 | 0.62 | 0.32-1.20 |
| Genus RuminococcaceaeUCG009 | 14 | IVW | 0.0166 | 1.47 | 1.07-2.01 |
| | 14 | MR Egger | 0.0373 | 4.28 | 1.27-14.46 |
| | 14 | Weighted median | 0.1074 | 1.43 | 0.93-2.20 |
| | 14 | Simple mode | 0.2089 | 1.74 | 0.76-3.98 |
| | 14 | Weighted mode | 0.8919 | 1.06 | 0.48-2.34 |
| Genus Streptococcus | 16 | IVW | 0.0044 | 1.94 | 1.23-3.05 |
| | 16 | MR Egger | 0.1450 | 3.90 | 0.69-21.99 |
| | 16 | Weighted median | 0.0344 | 1.97 | 1.05-3.69 |
| | 16 | Simple mode | 0.2422 | 1.94 | 0.67-5.61 |
| | 16 | Weighted mode | 0.1120 | 2.15 | 0.88-5.25 |
| Order Erysipelotrichales | 13 | IVW | 0.0384 | 1.77 | 1.03-3.03 |
| | 13 | MR Egger | 0.3025 | 3.62 | 0.35-37.31 |
| | 13 | Weighted median | 0.2702 | 1.51 | 0.73-3.13 |
| | 13 | Simple mode | 0.5926 | 1.39 | 0.43-4.45 |
| | 13 | Weighted mode | 0.5074 | 1.49 | 0.48-4.64 |

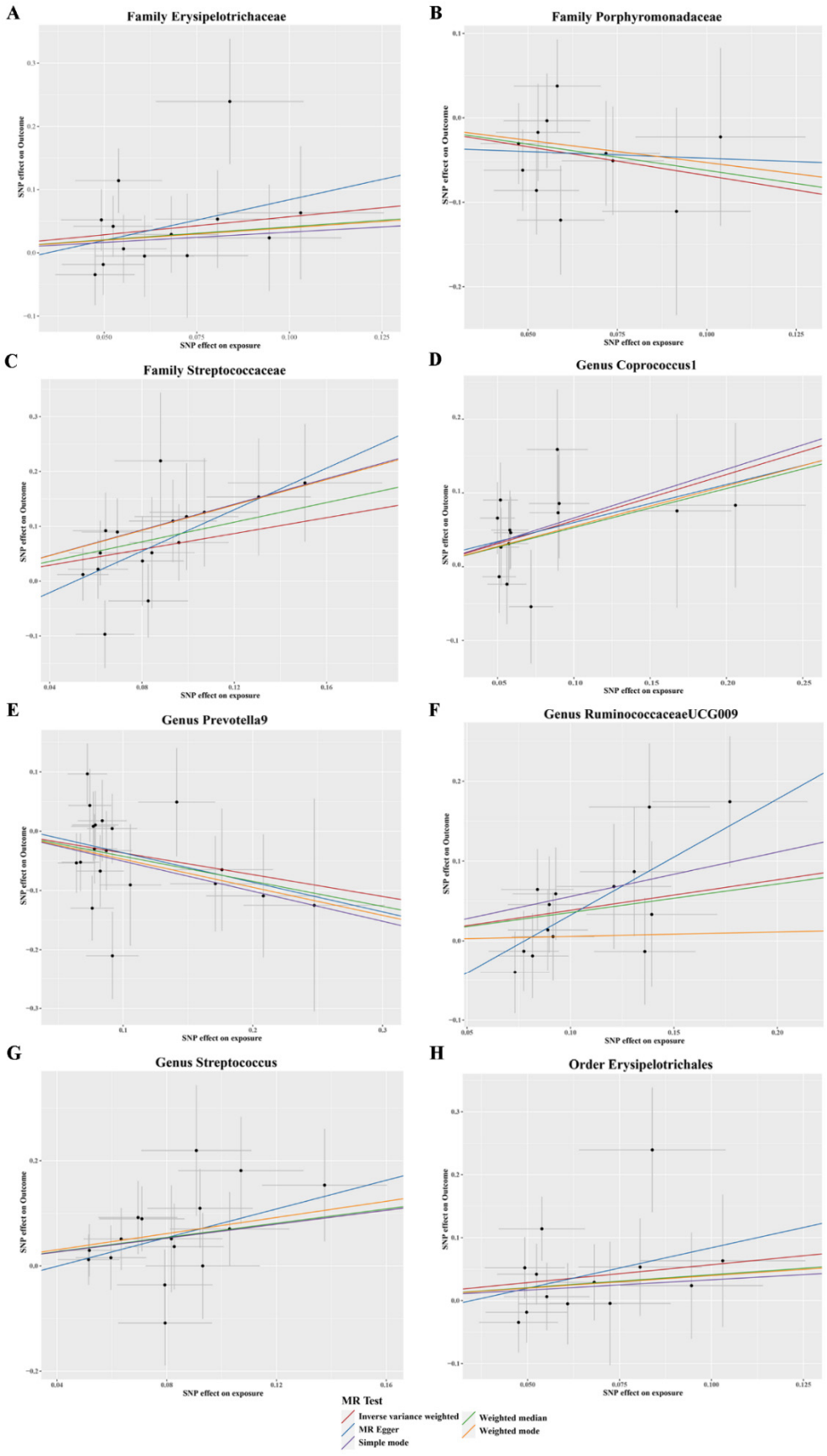



**Figure 1.** Scatter plots of Mendelian randomization analysis. The scatter plot of Erysipelotrichaceae (A), Porphyromonadaceae (B), Streptococcaceae (C), Coprococcus1 (D), Prevotella9 (E), RuminococcaceaeUCG009 (F), Streptococcus (G), Erysipelotrichales (H).

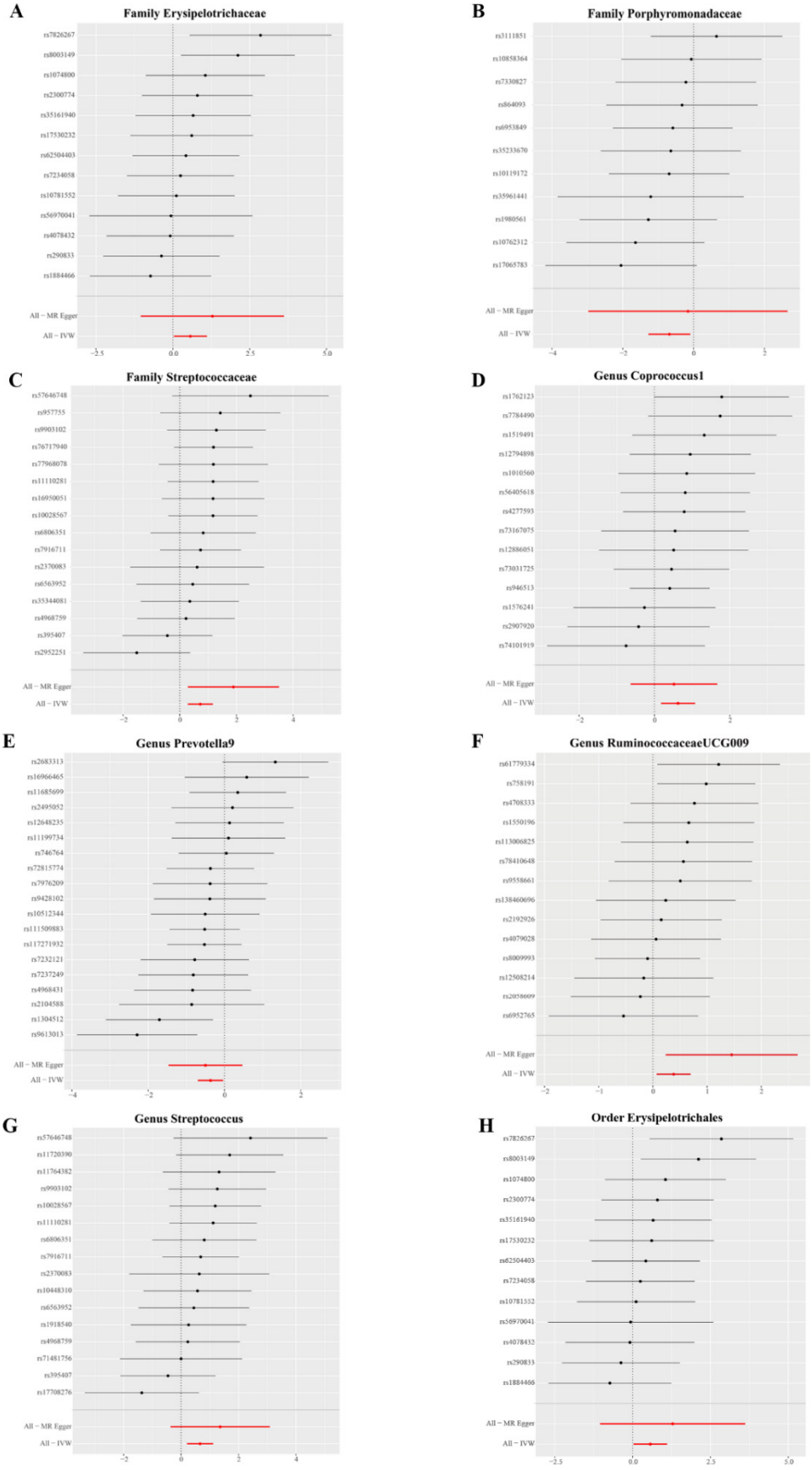



**Figure 2.** Forest plots of Mendelian randomization analysis. The forest plot of Erysipelotrichaceae (A), Porphyromonadaceae (B), Streptococcaceae (C), Coprococcus1 (D), Prevotella9 (E), RuminococcaceaeUCG009 (F), Streptococcus (G), Erysipelotrichales (H)

Our findings indicate that three family (Erysipelotrichaceae, Porphyromonadaceae, and Streptococcaceae), four genus (Coprococcus1, Prevotella9, RuminococcaceaeUCG009, and Streptococcus), and one order (Erysipelotrichales) of the gut microbiota have causal relationships with abnormal spermatozoa. IVW analysis suggested that Erysipelotrichaceae (OR=1.77, 95% CI:1.03-3.03), Streptococcaceae (OR=2.06, 95% CI: 1.33-3.21), Coprococcus1(OR=1.87, 95% CI: 1.19-2.94), RuminococcaceaeUCG009(OR=1.47, 95% CI: 1.07-2.01), Streptococcus (OR=1.94, 95% CI: 1.23-3.05), and Erysipelotrichales (OR=1.77, 95% CI: 1.03-3.03) increased the risk of abnormal spermatozoa. Meanwhile, Porphyromonadaceae (OR=0.50, 95% CI: 0.28-0.91) and Prevotella9 (OR=0.69, 95% CI:0.50-0.97) had a protective effect on cholelithiasis. (Table 2, **Figure 1**, and **Figure 2**)

## 3.3. Assessment of MR assumptions

Heterogeneity analysis results showed no statistically significant heterogeneity in all eight two-sample MR analyses ($p>0.05$) (Table 3). Leave-one-out analysis revealed no evidence of a significant effect of single SNPs on the overall impact (**Figure 3**). We found no significant horizontal pleiotropy from pleiotropy analysis results ($p>0.05$) (**Table 4**). The results of the MR-PRESSO analysis confirmed that there were no outliers in any of the eight two-sample MR analyses.

**Table 3.** Heterogeneity statistics of two-sample Mendelian randomization analysis.

| Bacterial Taxa (Exposure) | Methods | Q-value | Degrees of freedom | P-value |
|---|---|---|---|---|
| Family Erysipelotrichaceae | MR Egger | 9.9534 | 11 | 0.5346 |
| | IVW | 10.3369 | 12 | 0.5864 |
| Family Porphyromonadaceae | MR Egger | 5.5589 | 9 | 0.7831 |
| | IVW | 5.6990 | 10 | 0.8399 |
| Family Streptococcaceae | MR Egger | 9.7949 | 14 | 0.7770 |
| | IVW | 11.9862 | 15 | 0.6801 |
| Genus Coprococcus1 | MR Egger | 7.6347 | 12 | 0.8130 |
| | IVW | 7.6759 | 13 | 0.8641 |
| Genus Prevotella9 | MR Egger | 20.7514 | 17 | 0.2376 |
| | IVW | 20.8498 | 18 | 0.2871 |
| Genus RuminococcaceaeUCG009 | MR Egger | 6.2195 | 12 | 0.9046 |
| | IVW | 9.4061 | 13 | 0.7416 |
| Genus Streptococcus | MR Egger | 9.4315 | 14 | 0.8025 |
| | IVW | 9.4755 | 15 | 0.8514 |
| Order Erysipelotrichales | MR Egger | 9.9534 | 11 | 0.5346 |
| | IVW | 10.3369 | 12 | 0.5864 |

**Table 4.** Pleiotropy statistics of two-sample Mendelian randomization analysis.

| Bacterial Taxa (Exposure) | Methods | Egger_intercept | Standard Error | P-value |
|---|---|---|---|---|
| Family Erysipelotrichaceae | MR Egger | -0.0447 | 0.0723 | 0.5484 |
| Family Porphyromonadaceae | MR Egger | -0.0320 | 0.0856 | 0.7168 |
| Family Streptococcaceae | MR Egger | -0.0967 | 0.0653 | 0.1609 |
| Genus Coprococcus1 | MR Egger | 0.0084 | 0.0412 | 0.8426 |
| Genus Prevotella9 | MR Egger | 0.0133 | 0.0468 | 0.7799 |
| Genus RuminococcaceaeUCG009 | MR Egger | -0.1132 | 0.0634 | 0.0995 |
| Genus Streptococcus | MR Egger | 0.0163 | 0.0776 | 0.8369 |
| Order Erysipelotrichales | MR Egger | -0.0447 | 0.0723 | 0.5484 |

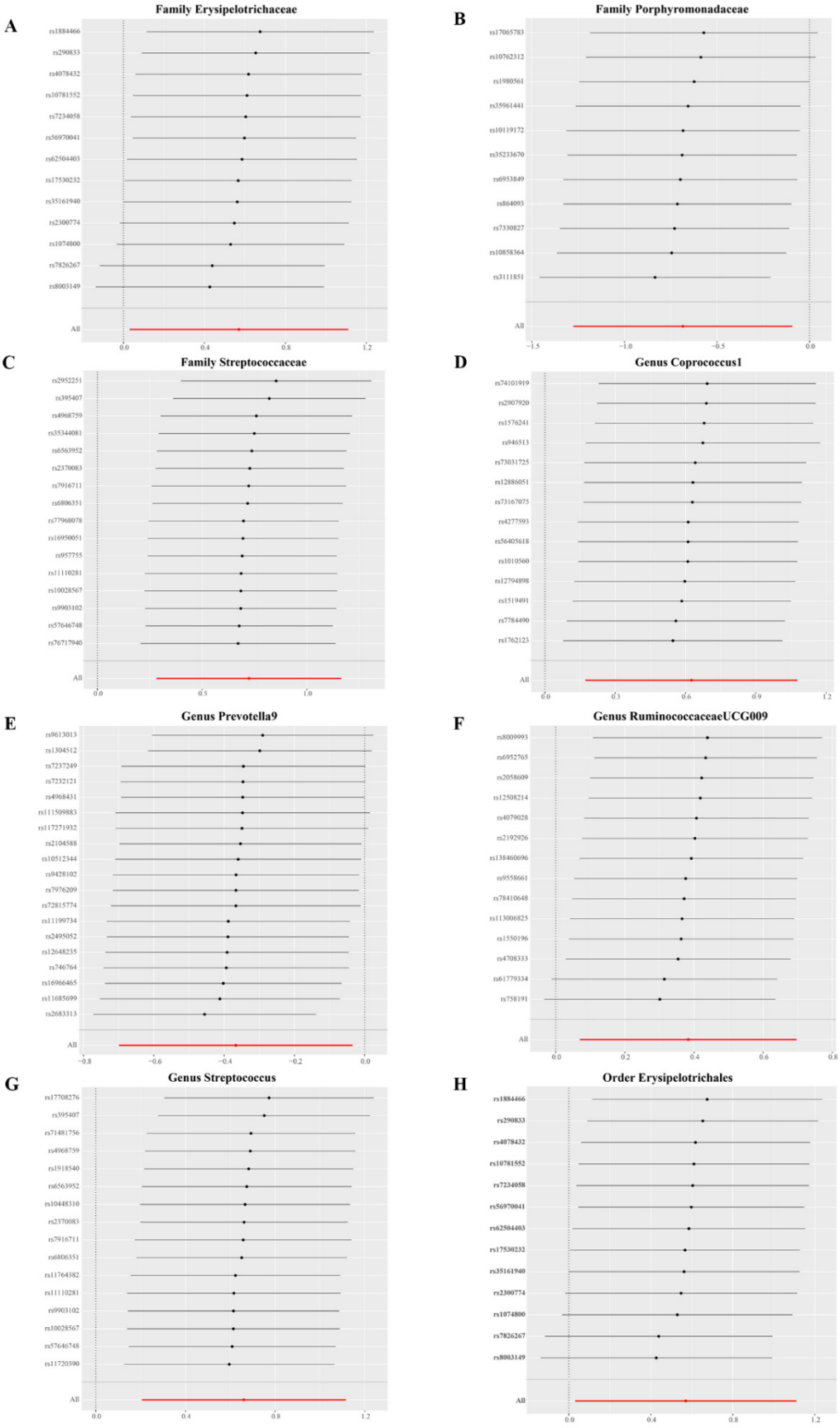



**Figure 3.** Leave-one-out sensitivity analysis. Leave-one-out sensitivity analysis of Erysipelotrichaceae (A), Porphyromonadaceae (B), Streptococcaceae (C), Coprococcus1 (D), Prevotella9 (E), RuminococcaceaeUCG009 (F), Streptococcus (G), Erysipelotrichales (H).

# 4. Discussion

This study explored the relationship between gut microbiota and abnormal spermatozoa using a two-sample Mendelian randomization analysis. Erysipelotrichaceae, Streptococcaceae, Coprococcus1, RuminococcaceaeUCG009, Streptococcus, and Erysipelotrichales increase the risk of abnormal spermatozoa. In addition, Porphyromonadaceae and Prevotella9 exhibited protective functions.

The human body contains trillions of symbiotic microorganisms necessary for host health, including bacteria, fungi, and viruses[22]. Gut microbiota can affect the quality of male Spermatozoa via nutrition, immunity, and signaling, which in turn affects fertility[4]. Vitamin A is an essential nutrient in supporting the reproductive system and facilitating embryonic development[24]. The lack of Vitamin A leads to the epithelia of the epididymis, prostate, and seminal vesicle being replaced with stratified squamous keratinizing epithelium, and spermatogenesis ceases[23, 24]. And gut microbiota is vital in regulating vitamin A absorption and metabolism[23, 24]. In a sheep model, disrupting the absorption of vitamin A from gut microbiota decreased the number of mature spermatozoa[26]. Gut microbiota like Bacteroides fragilis can produce vitamin K[25]; its ratio to vitamin D affects sperm development and motility[27]. In the fertilization process, calcium plays a crucial role[28]. Blood calcium levels are maintained by osteoclasts dissolving calcium salts in bone. In contrast, GM can regulate Ca2+ levels in the reproductive system by controlling the switch between blood and bone calcium[23, 29]. Dietary supplements and bacterial synthesis are the primary sources of folic acid[30]. Lactobacillus and Bifidobacterium are the most critical folic acid producers and metabolizers in gut microbiota[31]. Normal levels of serum folic acid positively affect semen parameters such as count, motility, and morphology[32].

It was reported that male mice fed purified lactic acid bacteria initially isolated from human milk exhibited larger testes and higher serum testosterone levels[33, 34]. Feeding old male mice purified microbes like Lactobacillus reuteri could restore testosterone to youthful levels[34, 35]. Hence, gut microbiota may play an essential role in regulating androgen metabolism[36]. Androgens, on the other hand, play a vital role in the normal process of spermatogenesis[37]. Recent studies have shown that gut microbiota plays a critical role in maintaining spermatogenesis and testicular health through the gut-testis axis[38]. Spermatogenesis can be improved or disrupted by different gut microbiota, according to some fecal microbiota transplantation studies[34, 39].

There have been several studies on the effect of gut microbiota on abnormal spermatozoa, but there are limitations to the studies. Firstly, many studies have often only been able to find a correlation between gut microbiota and abnormal spermatozoa but not to determine whether a causal relationship exists. This is because the biological relationship between abnormal spermatozoa and gut microbiota may be a complex one of mutual influence. Additionally, abnormal spermatozoa and gut microbiota composition may be influenced by environmental and lifestyle factors such as diet, substance use, and physical activity. These factors may be challenging to control in a study fully. They may, therefore, introduce confounding factors, making it difficult to determine a direct relationship between the microbiota and abnormal spermatozoa. Using the Mendelian randomization method to study the relationship between gut microbiota and abnormal spermatozoa is a powerful approach because it helps reduce the impact of confounding factors, thereby making the research results more reliable.

This study suggests the potential causal impact of gut microbiota on abnormal spermatozoa, and the several gut microbiota types identified in this study may hold promise for the prevention and treatment of abnormal spermatozoa.

# 5. Conclusion

Our research revealed a causal relationship between gut microbiota and abnormal spermatozoa. Erysipelotrichaceae, Streptococcaceae, Coprococcus1, RuminococcaceaeUCG009, Streptococcus, and Erysipelotrichales are promoters of abnormal spermatozoa. Porphyromonadaceae and Prevotella9 are protective factors for abnormal spermatozoa, which may provide clues for treating abnormal spermatozoa.

# Author Contributions

P.Q and L.H contributed to the study design and data acquisition, drafted the manuscript, and were the co-first authors. Prof. Zhang is considered correspondence author. All authors read and approved the final manuscript.

# Ethical Statement

This is a bioinformatics analysis; ethical approval is not required.

# Conflict of Interest

Not applicable.

# Data Availability

The datasets generated or analyzed in the current study will be available upon reasonable request.

# Conflict of Interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

# Abbreviations

| | |
|---|---|
| CI | Confidence interval |
| GWAS | Genome-wide association study |
| IVW | Inverse-variance weighted |
| MR | Mendelian randomization |
| MR-PRESSO | MR pleiotropy residual sum and outlier |
| OR | Odds ratio |
| SNP | Single nucleotide polymorphisms |

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