Original Article

A Mendelian randomization study on causal relationship between metabolic factors and abnormal spermatozoa

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Background: Male infertility is a global health problem. There is an increasing attention on the association of metabolic status with spermatogenesis. However, the impacts of metabolic factors on semen parameters are still unclear. To provide evidence for developing appropriate interventions on disease screening and prevention, we performed a Mendelian randomization (MR) analysis to assess causality between various metabolic factors and abnormal spermatozoa.

Methods: We conducted a two-sample MR study to appraise the causal effects of 16 metabolic factors (including indexes of metabolic traits, glucose metabolism, lipid profile, adipokines, uric acid and metabolic diseases) on abnormal spermatozoa from genome-wide association studies (GWASs). Filtering with strict criteria, eligible genetic instruments closely associated with each of the factors were extracted. We employed inverse variance weighted for major analysis, with supplement MR methods including MR-Egger and weighted median. Heterogeneity and pleiotropy tests were further used to detect the reliability of analysis.

Results: After rigorous quality control in this MR framework, we identified that body fat percentage [odds ratio (OR) =1.49, 95% confidence interval (CI): 1.01–2.20, P=0.046] and resistin (OR =1.55, 95% CI: 1.11–2.19, P=0.01) were causally associated with a higher risk of abnormal spermatozoa. In terms of other indexes of metabolic traits, glucose metabolism, serum lipid profile and uric acid and metabolic diseases including type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD), no causal effects were observed (P>0.05).

Conclusions: Our MR analysis provides robust evidence that body fat percentage and resistin are risk factors for abnormal spermatozoa, suggesting implications of identifying them for potential interventions and clinical therapies in male infertility. Further investigation in larger-scale GWASs on subgroups of abnormal spermatozoa will verify impacts of metabolic factors on spermatogenesis.

Keywords: Male infertility; Mendelian randomization (MR); spermatozoa; metabolism; resistin

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Introduction

Male infertility is a global health issue afflicting up to 12% of men worldwide, with a primary or contributing cause in approximately 50% of infertile couples (1,2). An updated systematic review confirms a severe decrease of 51.6% in testicular sperm production among men from various continents between 1973 and 2018. And this decline is even continuing in the 21st century at an accelerated pace globally (3). The dramatically increasing disease burden of abnormal spermatozoa reflects the decline in male fertility worldwide, which imposes psychological and social pressure on patients and weighs on economic burden of health-care systems (4). Research on the causes of abnormal spermatozoa is urgently needed to develop appropriate interventions for potential risk factors, to monitor access to quality fertility care, and eventually to mitigate the crisis in male reproductive health.

Previous observational or epidemiological studies on abnormal spermatozoa have put a spotlight on metabolic factors, including dietary habit, endocrine diseases and obesity epidemics (5,6). Further, researches on transgenerational transmission of epigenetic modifications suggest that state of impaired metabolism, such as obesity or diabetes, might have significant effects on male fertility and even compromise reproductive potential of offspring (7,8). However, since potential residual confounding and reverse causation arise the potential for spurious associations in those conventional studies, whether the associations of certain modifiable factors with the risk of abnormal spermatozoa are definitely causal remains undermined. In addition, there is still limited evidence determining causality between metabolic traits and semen parameters due to lack of rigorous randomized controlled trials (RCTs) designed and conducted with strict measured criteria (9,10).

Mendelian randomization (MR) utilizes specific genetic variants as instruments variables (IVs) in observational settings to strengthen causal inference between risk factors and disease outcomes (11). The random allocation of genetic variants at conception implies that the estimates through MR are less susceptible to confounding bias from environmental factors and reverse causality (11). This proves that MR is a reliable tool in the absence of RCTs to generate robust evidence and to seek risk factors for diseases. At present, there is still a lack of large-scale RCTs to infer the causality of metabolic factors with risk of abnormal spermatozoa. Hence, we conduct this MR study aiming to fill the gap in male fertility protection research and give rise to worldwide concern in this field. We present this article in accordance with the STROBE-MR reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-24-187/rc).

Highlight box

Key findings

- Body fat percentage and resistin are key risk factors for abnormal spermatozoa.

What is known and what is new?

- The impact of metabolic factors on spermatogenesis is still uncertain based on current research.
- Mendelian randomization is conducted to infer the association of multiple metabolic factors with risk of abnormal spermatozoa to fill the gap in male fertility protection research.

What is the implication, and what should change now?

- Large genome-wide association studies and randomized controlled trials are necessary to better reveal the cause-and-effect correlation between metabolic characteristics and spermatozoa.

Methods

Study design

A two-sample MR analysis was conducted in this study using publicly available genome-wide association studies (GWASs) summary-level data for 16 modifiable factors and abnormal spermatozoa.

The MR framework and analytic process conformed to the STROBE-MR guidelines (12), and the overview of study design is presented in *Figure 1*. All the original GWASs summary datasets cited in our study were publicly available and had been approved by their corresponding ethical review committees respectively; therefore, no separate ethical approval was required for this study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Genetic instrument selection

The selection of genetic instrument was based on three critical assumptions: (I) Assumption 1 is the relevance assumption that the genetic variants used as IVs should be highly related to the exposure; (II) Assumption 2 is the independence assumption that the genetic variants for the exposure should not be associated with any confounders in the causality between the exposure and outcome; (III)

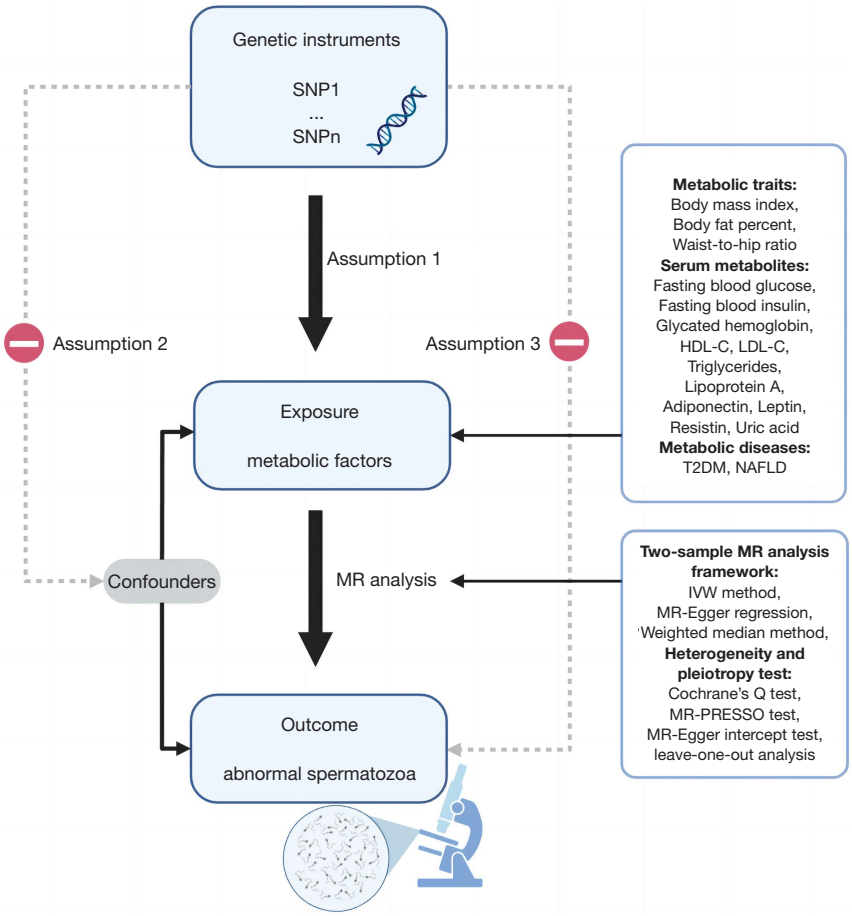

Figure 1 MR study design overview (detailed assumptions can be found in the method of genetic instrument selection); HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T2MD, type 2 diabetes mellitus; NAFLD, non-alcoholic fatty liver disease; SNP, single-nucleotide polymorphism; MR, Mendelian randomization; IVW, inverse variance weighted; MR-PRESSO, MR-Pleiotropy Residual Sum and Outlier methods.

Assumption 3 is the exclusivity assumption that the genetic variants affect the outcome merely through their effects on the exposure, rather than draw a direct connection to the outcome.

The selection of metabolic factors in this MR study was based on their clinical applicability. These factors are closely linked to metabolic syndrome, but the role of them was controversial in recent studies and lack of RCTs to confirm their causality with abnormal spermatozoa. Through evaluating these factors, we aimed to indicate the significance of lipid, glucose, and purine metabolism within the testicular environment and the impact on sperm quality, which might help elucidate the influence of metabolic factors on male reproductive health and contribute valuable insights to the emerging field of interest in reproductive biology. The 16 metabolic factors included body mass index, body fat percent, waist-to-hip ratio, fasting blood glucose, fasting blood insulin, glycated hemoglobin, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, lipoprotein A, adiponectin, leptin, resistin, uric acid, type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD) (13-20).

Following the prerequisite mentioned above, single-nucleotide polymorphisms (SNPs) strongly associated at the genome-wide significance level ($P \leq 5 \times 10^{-8}$) with the metabolic factors above were extracted from relevant GWASs (*Table 1*). Then, we excluded those SNPs in high linkage disequilibrium and remained the independent SNPs ($r^2 < 0.001$) as IVs, which was estimated based on the 1000

Table 1 Detailed information on data sources of exposures

Exposure or outcome	Unit	Participants	IVs	F statistics	Consortium or study	PubMed ID
Exposure						
Body mass index	SD (kg/m^2)	499,393 European	128	21,456	UK Biobank	–
Body fat percentage	SD (%)	492,781 European	127	33,142	UK Biobank	–
Waist-to-hip ratio	SD	93,478 European	5	271	GIANT	25673412
Fasting blood glucose	SD (mmol/L)	200,622 European	61	6,194	A study	34059833
Fasting blood insulin	SD (pmol/L)	151,013 European	38	5,126	A study	34059833
Glycated hemoglobin	SD (%)	146,806 European	69	10,287	A study	34059833
HDL-C	SD (mmol/L)	432,018 European	141	28,753	UK Biobank	–
LDL-C	SD (mmol/L)	469,878 European	74	11,201	UK Biobank	–
Triglycerides	SD (mmol/L)	470,346 European	114	31,901	UK Biobank	–
Lipoprotein A	SD (nmol/L)	377,555 European	9	1,318	UK Biobank	–
Adiponectin	Ln (mg/dL)	39,883 European	14	925	ADIPOGen	22479202
Leptin	Log (ng/mL)	49,909 European	3	134	A study	32917775
Resistin	SD (ng/mL)	21,758 European	13	221	A study	33067605
Uric acid	SD (mg/mL)	343,836 European	6	1,269	A study	34594039
T2DM	OR	61,714 cases and 593,952 controls of European ancestry	114	31,511	A study	30054458
NAFLD	OR	8,434 cases and 770,180 controls of European ancestry	4	254	A study	34841290
Outcome						
Abnormal spermatozoa	OR	1,913 cases and 293,878 controls of European ancestry	–	–	FinnGen	–

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T2DM, type 2 diabetes mellitus; NAFLD, non-alcoholic fatty liver disease; IVs, instruments variables; SD, standard deviation; OR, odds ratio.

Genomes European reference panel (21). Moreover, all these selected genetic instruments mentioned above should have strong potential to predict abnormal spermatozoa with *F* statistic greater than 10 (22). After filtering with these strict criteria, the remaining SNPs were regarded as eligible IVs. Detailed information on data sources of exposures including number of participants and IVs is shown in *Table 1*.

Data source of outcome

We obtained the GWAS summary data for abnormal spermatozoa from FinnGen Consortium R7 which was publicly released in 2022. The diagnostic criteria for disease were based on laboratory findings, including sperm count, vitality and morphology. Totally, the cohort of abnormal spermatozoa included 1,913 cases and 293,878 controls. The exposure and the outcome cohorts were both from European ancestry individuals to avoid violation caused by population differences (22). We retrieved summary data from the FinnGen, and extracted SNPs associated with metabolic status (including the effects of each of the SNPs on abnormal spermatozoa, beta coefficients and standard errors).

Statistical analysis

After harmonization to omit palindromic and incompatible SNPs across the GWASs of exposure and outcome data, we employed several MR approaches to run MR estimates of metabolic factor for abnormal spermatozoa, namely

the inverse variance weighted (IVW), weighted median and MR-Egger. Multiple approaches stated above were on the basis of their different underlying assumptions for horizontal pleiotropy (violation of the exclusion restriction assumption that SNPs affect abnormal spermatozoa not merely through the exposure). As the primary statistical analysis, IVW is a method of weighting averages of random variables, where instruments can affect the outcome only through the exposure of interest and not by any alternative pathway (23). Exceptionally, when unbalanced horizontal pleiotropy exists, the selected SNPs might be invalid IVs in the IVW meta-analysis. Therefore, the MR-Egger and weighted median methods were further used to supplement IVW estimates as they could provide more robust estimates to the results in a broader set of scenarios but with relatively less efficiency. The MR-Egger can generate corrected MR estimates after adjustment for pleiotropic effects (24). The weighted median method is capable of consistent estimates if more than 50% of the genetic instruments are valid (25).

Sensitivity analysis has been essential in MR studies to detect potential horizontal pleiotropy and the heterogeneity. Firstly, Cochrane Q value was used as a marker of their pleiotropic effects to assess heterogeneity among SNP estimates (23). Secondly, we obtained the intercept test from the MR-Egger regression as an indicator of directional pleiotropy ($P<0.05$ was considered as the existence of directional pleiotropy (24). Moreover, we performed MR-Pleiotropy Residual Sum and Outlier methods (MR-PRESSO) to identify the outlier variants in MR estimates, remove them and correct horizontal pleiotropy (26). Eventually, leave-one-out analysis was also employed to evaluate whether the MR evaluation might be biased by some single SNP.

The above results were reported as the odds ratios (ORs) and 95% confidence intervals (CIs). We also adopted two-sided P values, and regarded P values less than 0.05 as suggestive significance. All the MR analyses were conducted using the R software (version 4.3.1) with the R package "TwoSampleMR", "MRPRESSO", and "forestplot".

Results

The detailed information of data sources for the genetic instruments and the numbers of valid SNPs for each of 16 exposures in this present study are presented in *Table 1*. All *F* statistics for the overall instruments were more than 10, indicating the qualified power of the available genetic instruments.

In terms of three metabolic traits, the primary analysis IVW indicated that genetically predicted one standard deviation (SD) increase in body fat percentage might be causally associated with a higher risk of abnormal spermatozoa (OR =1.49, 95% CI: 1.01–2.20, P=0.046), while no causal effect was observed for body mass index (BMI) (OR =1.91, 95% CI: 0.73–5.01, P=0.19), and waist-to-hip ratio (OR =0.09, 95% CI: 0.00–697.20, P=0.60) (*Figure 2*).

For the 11 serum metabolites including three glucometabolic related parameters, four lipid parameters, and three kinds of adipokines and uric acid, we observed a causal relationship between resistin and higher risk of abnormal spermatozoa (OR =1.55, 95% CI: 1.11–2.19, P=0.01). However, there was no significant potential association between fasting blood glucose, fasting blood insulin, glycated hemoglobin, HDL-C, LDL-C, triglycerides, lipoprotein A, adiponectin, leptin and uric acid and abnormal spermatozoa (*Figure 2*).

For T2DM (OR 0.99, 95% CI: 0.89–1.10, P=0.88) as well as NAFLD (OR =0.97, 95% CI: 0.79–1.19, P=0.75), no causal relationship was found (*Figure 2*). Meanwhile, the weighted median analysis and the sensitivity analyses including the test of heterogeneity and pleiotropy supported the above causation between metabolic factors and abnormal spermatozoa, which are presented in *Table 2*.

Discussion

In this population-based MR study, we investigated the causal effects of 16 metabolic factors on abnormal spermatozoa. Our findings suggested that genetically determined increased body fat percent and resistin were correlated with a higher risk of abnormal spermatozoa. In terms of other metabolic factors including BMI, waist-to-hip ratio, fasting blood glucose, fasting blood insulin, glycated hemoglobin, HDL-C, LDL-C, triglycerides, lipoprotein A, adiponectin, leptin, uric acid, T2DM and NAFLD, no causal effects were observed.

The association between obesity and infertility has been extensively followed with interest as the global epidemic of obesity rises sharply. However, the impact of overweight and obesity on semen parameters is still inconclusive. Although the first systematic review and meta-analysis concluded that the observed effects of obesity on sperm concentration were not significant (27), some updated systematic reviews have leaned toward the viewpoint that overweight and/or obesity categories might be associated with lower sperm quality

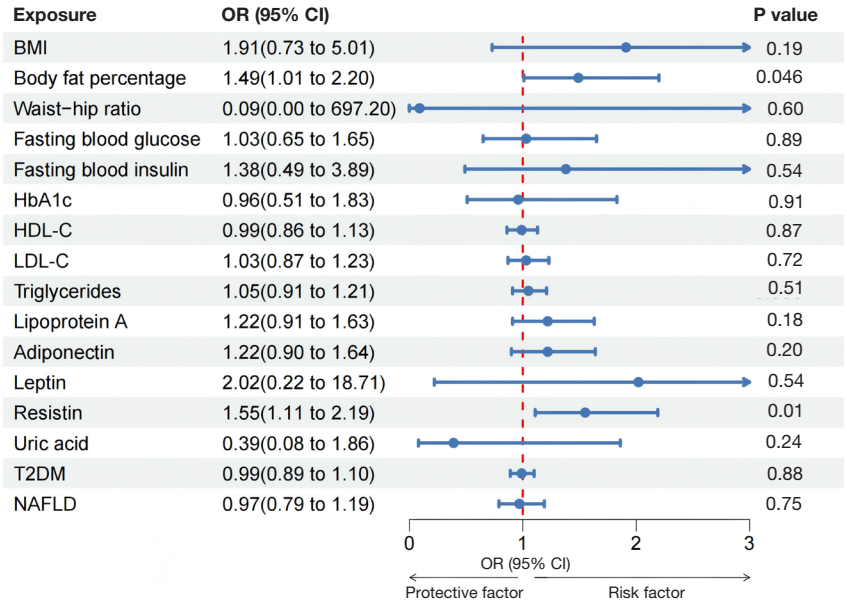

Figure 2 The effect estimates of metabolic exposures on abnormal spermatozoa. BMI, body mass index; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T2DM, type 2 diabetes mellitus; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; CI, confidence interval.

Table 2 Test of heterogeneity and pleiotropy and weighted median method of metabolic factors on abnormal spermatozoa

Exposure	Test of heterogeneity		Test of pleiotropy		P value (weighted median method)
	Cochrane Q test	P value (heterogeneity)	MR-Egger intercept	P value (pleiotropy)	
Body mass index	139.993	0.20	–0.028	0.17	0.69
Body fat percentage	111.890	0.81	0.012	0.51	0.08
Waist-to-hip ratio	10.335	0.28	–0.383	0.57	0.34
Fasting blood glucose	75.899	0.08	–0.006	0.53	0.78
Fasting blood insulin	67.573	0.19	–0.030	0.29	0.33
Glycated hemoglobin	79.553	0.16	0.001	0.90	0.41
HDL-C	115.180	0.94	0.008	0.94	0.18
LDL-C	75.970	0.38	–0.002	0.81	0.97
Triglycerides	115.848	0.41	–0.002	0.87	0.15
Lipoprotein A	4.434	0.82	0.000	0.99	0.32
Adiponectin	15.121	0.30	0.000	0.99	0.18
Leptin	10.898	0.43	0.478	0.21	0.41
Resistin	9.936	0.62	0.016	0.64	0.06
Uric acid	228.551	0.81	–0.002	0.70	0.45
T2DM	134.540	0.08	–0.001	0.93	0.76
NAFLD	2.579	0.46	0.001	0.99	0.84

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MR, Mendelian randomization; T2DM, type 2 diabetes mellitus; NAFLD, non-alcoholic fatty liver disease.

including semen volume, sperm count and concentration, sperm vitality, total motility and normal morphology (28,29). Obesity might affect sperm directly or indirectly through several probable mechanisms including alterations in male sexual hormone profile (30), increased production of reactive oxygen species (ROS) and inflammatory mediators (31,32), and interaction of adipose tissue on testicular temperature inducing adverse living environment for sperm (33), and epigenetic changes including DNA methylation reprogramming and modification of noncoding RNAs in sperm (34,35). In our MR analysis, we employed three measured markers related to obesity, one of which namely body fat percent showed a harmful effect on sperm, but with weak statistical significance. Our finding supports the suggestion that obesity affects sperm quality, but the causal mechanism between obesity and spermatogenesis and sperm maturation is still unclear, and further research should be carried on to confirm this relationship in the future.

With regard to lipid metabolism, which plays a crucial role in spermatogenesis and obesity, its fluctuations in serum lipid metabolites might be a sign of abnormal spermatozoa. In light of the current knowledge, no studies have utilized MR to reveal a causal relationship between level of lipid metabolites and semen parameters. Therefore, the positive result of resistin brings a new insight that circulating levels of some serum adipokines that are less commonly used in clinical practice, might be independent risk factors for abnormal spermatozoa. Resistin is a 12.5 kDa pro-inflammatory adipokine that circulates in human blood as a dimeric protein (36). In the reproductive system, it is mainly expressed in rat Leydig cells, Sertoli cells and macrophages where it might regulate testicular functions under physiological condition, but this has not been demonstrated in humans (37). Recent researches reported that there are two putative binding sites for resistin: adenylyl cyclase-associated protein 1 (CAP1) and toll-like receptor 4 (TLR-4), which have been found in human sperm, and it is acknowledged that resistin activates various signaling pathways in different tissues such as Akt, MAPK, STAT3 and peroxisome proliferator-activated receptor gamma (PPARγ) (38,39). Nevertheless, results of previous studies upon the relationship between resistin and semen are heterogeneous. Moretti *et al.* found that concentration of resistin in seminal plasma was negatively correlated with sperm motility, and this adipokine is associated with markers of inflammation in seminal plasma such as elastase, interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) (40). When inflammation occurs, the levels of these cytokines and ROS increase, which accounts for the impaired process of spermatogenetic present in human testis and induces a decrease in spermatic concentration, motility, and sperm count (40,41). However, two other researches did not show significant correlation between resistin concentrations in seminal plasma and sperm parameters (42,43). Given the small sample sizes and the low number of studies available, it is difficult to draw a definite conclusion through these researches. Indeed, based on MR, our study removed confounding factors to ensure that circulating resistin is independent of any body parameter and provided strong support for conclusion that the presence of this adipokine would be related to an alteration of sperm parameters. Furthermore, it is still necessary to elucidate specific mechanism of action of resistin and clarify the intertwined relationship between resistin and spermatozoa.

Although we found that the resistin measurement in serum among lipid biomarkers appeared to be robust risk factor for abnormal spermatozoa, there was lack of a causal association between other lipid parameters or adipokines and sperm quality. What account for this contradictory result might be the complex etiology between lipids and sperm production, which also leads to the conflict in the current epidemiology researches or observational cohort studies on the relationship between them (44). The proportion of lipids, such as cholesterol, present in sperm membranes has close relationship with the sperm morphology and fertility potential (45). The lipid in the sperm membrane, as one of the raw materials to maintain the stability of the cell membrane or as chemical messengers between cells, is essential to a certain extent with its physiological variation of content throughout the process of sperm differentiation, sperm maturation, capacitation, and acrosome reaction (45,46). However, it seems that the lipids in seminal plasma might be derived from epithelial cells in the male reproductive tract rather than blood (47). Taking the reverse into consideration, most previous researches suggested that in the case of increased lipid profile, decreased testosterone with leptin resistance, excess oestrogen can cause disturbances in spermatogenesis, apoptosis (abortive), and sperm damage (30,48,49). In addition, excessive blood lipid also involves an increase in ROS and breaks the balance of antioxidant system, so that the decline of count, morphological deterioration and DNA fragmentation of sperm would eventually occur (31). While our results suggested that such associations between lipid profile and abnormal spermatozoa might not be directly

causal, and more comprehensive mechanism researches are necessary to further confirm their relationship.

Another important factor that might affect male fertility is purine metabolism. Uric acid is an important product related to purine nucleotide metabolism, which is the backbone of DNA molecules in sperm, and serum uric acid were highly correlated with testosterone levels (50). Some studies noted that a specific concentration of uric acid in semen can effectively sustain and enhance sperm motility and morphology, while also protecting functional integrity of sperm through the neutralization of oxidation processes, including endogenous free radicals and exogenous toxins (51). In contrast, various researches have consistently demonstrated that elevated levels of uric acid can exert a detrimental impact on sperm function such as the fertilization rate, to some extent, by diminishing the activity of crucial enzymes within sperm (52,53). However, our study did not find a direct causal relationship between serum uric acid and abnormal spermatozoa, therefore the determination of uric acid in the clinic needs further discussion in the management of infertility.

T2DM and NAFLD are metabolic diseases related to abnormal spermatozoa. Current cross-sectional studies investigating semen parameters and men with diabetes mellitus are heterogeneous. Over the decades, several cohort studies have identified associations between T2DM and increased risk of reduced sperm count and motility and increased morphological abnormalities (50,54). Characteristics of T2DM include abnormal glucose and lipid metabolism, which might progress to hyperinsulinemia and insulin resistance. These changes can not only induce disturbances in endocrine control and dysregulated spermatogenesis but also affect the sperm maturation process by increasing the substantial implications in the sperm DNA/chromatin levels of diabetes patients (55,56). On the contrary, there are meta-analyses supporting a negative effect of diabetes on sperm morphology but no effect on sperm count, with contradictory results concerning other semen parameters (57,58), which might be consistent with our conclusion that no significant causal effects were observed between T2MD (including several glycemic traits) glycemic traits and semen analysis. As for NAFLD, it is well known that NAFLD is closely linked to rise in the prevalence of diabetes, obesity, and male infertility. In contrast, the causal relationship between NAFLD and abnormal spermatozoa is still unclear, with a few studies being reported among relatively small cohorts (59,60). An early case-control study showed that NAFLD could significantly affect sperm concentration, count and total motility instead of semen volume and morphology (60), and NAFLD impairs reproductive function in male rats by decreasing the synthesis of testicular testosterone (61). In this MR study, we found no strong evidence to support associations between T2DM, NAFLD and abnormal spermatozoa, which is not in line with observational studies (55,60), these null findings should be cautiously interpreted given high heterogeneity in these analyses as well as a few genetic instruments for the metabolic diseases. Thus, the effects of T2DM and NAFLD on metabolic profiles need to be further explored.

The major strength of our present study lies in the first utilization of genetic instruments as proxies for various metabolic factors to perform two-sample MR analysis to infer their causal effects on risk of abnormal spermatozoa. After rigorous IVs selection and sensitivity testing, MR paradigm minimizes environmental confounding, diminishes reverse causality and derives robust evidence for causal effects of these risk factors, rather than just an association provided by most current cross-sectional studies, which might also be modified by the development and progression of the disease. In addition, we confined our analysis to the large-scale population of European ancestry, which effectively minimize the bias caused by the population structure.

Limitations of this MR investigation are also taken into account. Firstly, there is a lack of more detailed description upon the severity of sperm in patients with abnormal spermatozoa (regarding substantial parameters such as count, motility, malformation rate and DNA fragmentation index, etc.) in Finngen GWAS database or other available GWAS data of consistent race; thus, hierarchical analyses of different types of abnormal spermatozoa were unable to be conducted. A broader study containing subgroups of abnormal spermatozoa can be considered in the future to confirm the effects of metabolism on various types of abnormal sperm parameters more precisely. Secondly, the small sample size of GWASs might contribute to imprecision in the selection of genetic instruments, which could result in insufficient power to detect small or moderate associations. To solve these faults, larger GWASs and more well-designed clinical trials are necessary to better reveal the cause-and-effect correlation and to evaluate interventions targeting body fat or resistin. It is also indispensable to conduct the investigation of specific mechanisms by which resistin affects sperm through *in-vitro* and *in-vivo* experiments. Another limitation is that our

study population of consistent ancestry might obstruct the generalizability of our findings to other populations, which is also urged to be solved in the larger-scale MR study in the future.

Conclusions

In conclusion, this is the first wide angled MR analysis to explore the causality from metabolic factors on abnormal spermatozoa. Our MR analysis provides suggestive evidence that body fat percentage and resistin are risk factors for abnormal spermatozoa, but does not support the causal impact of other metabolic factors including BMI, glucose parameters, lipid profile, uric acid, T2DM and NAFLD. Further MR study with more genetic instruments for metabolic risk factors and diseases, followed by the investigation in larger-scale GWASs on subgroups of abnormal spermatozoa in the future, are necessary to confirm our findings.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the STROBE-MR reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-24-187/rc

Peer Review File: Available at https://tau.amegroups.com/article/view/10.21037/tau-24-187/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-24-187/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are 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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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