Enterotypes in asthenospermia patients with obesity

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Article

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Abstract

The essence of enterotypes is stratifying the entire human gut microbiome, and enterotypes modulates the association between diet and disease risk.We designed a study at the Center of Reproductive Medicine, Shengjing Hospital of China Medical University and Jinghua Hospital of Shenyang. *Prevotella* and *Bacteroides* were measured in stool samples from 178 men with enterotype B (61 normal, 117 overweight/obese) and 229 men with enterotype P (74 normal, 155 overweight/obese). The ratio between *Prevotella* and *Bacteroides* abundance, P/B, was used as a simplified way to distinguish the predominant enterotype. In enterotype P, obesity was a risk factor for decreased rate of forward progressive sperm motility (odds ratio [OR] 3.350; 95% confidence interval [CI] 1.881–5.966; P<0.001) and decreased rate of total sperm motility (OR 4.298; 95% CI 2.365–7.809; P<0.001). Obesity was also an independent risk factor (OR 3.131; 95% CI 1.749–5.607; P<0.001) after adjusting for follicle-stimulating hormone. In enterotype P, body mass index (BMI), as a diagnostic indicator of decreased rate of forward progressive sperm motility and decreased rate of decreased total sperm motility, had area under the curve values of 0.627 (P = 0.001) and 0.675 (P<0.0001), respectively, that were significantly higher than the predicted values in all patients. However, obesity was not a risk factor for asthenospermia, and there was no significant difference between obesity and sperm quality parameters in men with enterotype B (P>0.05). Our findings are the first to introduce enterotypes as a population-based individualized classification index to investigate the correlation between BMI and asthenospermia. In our study, overweight/obese men with enterotype P were found to have poorer sperm quality; however, sperm quality was not associated with overweight/obese in men with enterotype B. Besides, BMI is a risk factor for asthenospermia only in men with enterotype P but not in men with enterotype B.

Introduction

Nowadays, it is generally accepted that asthenospermia, which leads to male infertility, is a complex disease in which various etiologic factors are involved. In addition to genetic factors, lifestyle and environment also affect sperm motility. In recent decades, the global obesity epidemic has paralleled a decrease in semen quality (Chen et al.,2020;Kahn et al.,2017;Leisegang et al.,2021;Liu et al.,2017;Ma et al.,2019;Skoracka et al.,2020). Therefore, obesity is considered to be a lifestyle factor that may adversely affect semen quality. Recent studies have investigated the associations between obesity and semen motility, but the results remain inconsistent (Chen et al.,2020;Duits et al.,2010;Ma et al.,2019;Sermondade et al.,2012;Skoracka et al.,2020;Yang et al.,2021). For example, Ma et al. reported that obesity was substantially associated with a 3.6% (0.2%, 6.9%) reduction in total motile sperm count (Ma et al.,2019). Nathalie et al. also suggested that overweight and obese men were at significantly increased odds of presenting with azoospermia compared to men of normal body weight (Sermondade et al.,2012). In contrast, a prospective cohort study demonstrated that semen quality was not statistically significantly affected by body mass index (BMI) in a cohort of male partners in subfertile couples (Duits et al.,2010). Taken together, the current evidence on the association between BMI and semen quality is inconclusive. It can be speculated that that the population-based individualized differences may be involved in the effect of BMI on sperm motility.

Gut microbiota is recognized as the second genome of the human body that plays a variety of roles in health and disease affected by dietary structure. Studies have shown that a high-fat diet may lead to decreased sperm quality through alterations in intestinal microbiome(Ding et al.,2020;Yang et al.,2020), and that modulating gut microbiota may improve sperm quality(Zhang et al.,2021). Enterotype is a classification of gut microbiota in different populations, indicating the variation of gut microbiota is stratified between individuals, rather than continuous.Studies have shown that people with different enterotypes may have different responses to disease triggers(Arumugam et al., 2011).However, the relationship between enterotype, asthenozoospermia, and BMI was not clearly demonstrated till now. In our previous researches, we found a positive relationship between asthenozoospermia and BMI, but the association was not stable in all cases. In this study, experiments were designed to investigate the association of asthenozoospermia and BMI in men with different enterotypes.

Results

Determination of enterotypes according to the P/B ratio

Gut microbiota analysis was performed on the stool samples of all participants (n=407). The frequency plot of the relative abundance of log (P/B) (Figure 1) demonstrated a bimodal distribution of separation between the two groups. These results suggested that the participants could be classified into two groups: enterotype P (P/B ≥0.01) and enterotype B (P/B <0.01).

Clinical parameters

The baseline characteristics, sperm quality parameters, and sex hormone data of 407 patients enrolled in this study are listed in Table 1. Patients were divided into normal (BMI<24 kg/m^2) group and overweight/obese (BMI≥24 kg/m^2) group according to their BMI. The baseline characteristics, sperm quality parameters, and sex hormone data of the two groups are shown in Table 2. The levels of rate of forward progressive sperm motility, rate of total sperm motility, and TT were different between the two groups. In the normal BMI group, there were more patients with normal rate of forward progressive sperm motility and total sperm motility, whereas in the overweight/obese group, there were more patients with low rate of forward progressive sperm motility and total sperm motility, and the difference was statistically significant. However, there were no statistically significant differences in total sperm count and sperm concentration between the two groups.

Logistic regression analysis of risk factors for asthenospermia

As shown in Table 3, the incidence of asthenospermia in the overweight/obese group was significantly higher than that in the normal group (P=0.004). Univariate logistic regression showed that decreased rate of forward progressive sperm motility (OR 1.849; 95% confidence interval [CI] 1.218–2.807; P = 0.004) and decreased rate of total sperm motility (OR 2.406; 95% CI 1.346–3.111; P = 0.001) were significantly associated with obesity, as evident from the data of the overweight/obese group. Second, multivariate logistic regression analysis confirmed that obesity (OR 1.793; 95% CI 1.177–2.729; P = 0.006) was a risk factor for the development of asthenospermia after adjusting for FSH (Table 3). FSH was identified as a protective factor for asthenospermia.

Obesity is a risk factor for asthenospermia under enterotype P

As stated, obesity is significantly associated with decreased sperm quality. Considering that enterotypes modulate the risk of disease, we evaluated the effect of BMI on sperm quality in patients with enterotypes P and B.

First, rate of forward progressive sperm motility (Figure 2A), total sperm motility (Figure 2B), sperm concentration (Figure 2C) and total sperm count (Figure 2D) decreased with increasing BMI in patients with enterotype P but not significantly in patients with enterotype B (Figure 2E, F, G and H). Specifically, as shown in Table 4, in men with enterotype P, obesity was a risk factor for decreased rate of forward progressive sperm motility (OR 3.350; 95% CI 1.881–5.966; P<0.001) and decreased rate of total sperm motility (OR 4.298; 95% CI 2.365–7.809; P<0.001). On the contrary, in patients with enterotype B, no significant difference between obesity and sperm motility was found (Supplementary Table 2). Second, in enterotype P, obesity was still an independent risk factor (OR 3.131; 95% CI 1.749–5.607; P<0.001) after adjusting for FSH (Table 4).

Prediction Performance of enterotypes for categorizing asthenospermia

As mentioned above, in the enterotype P group, the increase in BMI was strongly associated with decreased sperm motility. Thus, using ROC curves, we further confirmed the correlation between BMI and asthenospermia in patients with enterotype P. In enterotype P, BMI resulted in higher area under the curve (AUC) of 62.7% and moderate sensitivity (78.8%) and highest specificity (49.5%) for decreased rate of forward progressive sperm motility (Figure 3A). Similarly, the BMI levels also resulted in the higher AUC of 67.5%, with sensitivity (81.1%) and highest specificity (52.3%) for decreased rate of total motility in men with enterotype P (Figure 3B). In contrast, BMI showed poor prediction performance for asthenospermia in men with enterotype B (Figure 3A and B).

Discussion

To the best of our knowledge, our study is the first to use enterotypes as a population-based individualized classification index to investigate the correlation between BMI and asthenospermia. We observed a correlation between obesity and the risk of asthenospermia under enterotype P. In this enterotype group, obesity was associated with poor sperm motility. However, in enterotype B group, sperm motility was not associated with obesity. Our results also showed that BMI could predict the risk of asthenospermia in the enterotype P group but not in the enterotype B group.

The effect of obesity on sperm motility is controversial. In a series of studies in Asian population, the results tend to be consistent; that is, obesity has a negative impact on sperm quality (Ma et al.,2019;Ramaraju et al.,2018;Sekhavat et al.,2010). In a study on Chinese sperm donors, obesity was found to be significantly associated with a 4.2%, 3.9%, and 3.6% reduction in semen volume, total sperm count, and total motile sperm count, respectively (Ma et al.,2019). A cross-sectional study in Iran showed that total sperm count and sperm motility in overweight and obese men were significantly lower than those in men with normal BMI (Sekhavat et al.,2010).

In a study based on data from infertility clinics in India, Ramaraju et al. (Ramaraju et al.,2018) found that obese men were more likely to have asthenospermia and oligospermia. In contrast, several studies in Europe have shown that men with excess body weight do not have considerable sperm motility problems (Aggerholm et al.,2008;Duits et al.,2010;M et al.,2014;Thomsen et al.,2014). A prospective cohort study in Netherlands showed that semen quality was not statistically significantly affected by BMI in male partners in subfertile couples (Duits et al.,2010). Similar results were also described in Danish and Austrian populations (Aggerholm et al.,2008;M et al.,2014;Thomsen et al.,2014). People with different enterotypes may have distinct digestive functions (Wu et al., 2011). Enterotype B is more common in in people who eat protein and fat as their long-term diets, such as western subjects, whereas enterotype P is more common in people who take carbohydrates as their long-term diets and is more often associated with non-Western subjects such as Asians (Gorvitovskaia et al.,2016). Our study showed that in enterotype P, obesity was a risk factor for decreased rate of forward progressive sperm motility and decreased rate of total sperm motility. We speculate that people with P enterotype should have a lower BMI, and their increased weight may indicate a dysbiosis of the gut microbiota, which may be one of the reasons for the decline in sperm quality. In conclusion, the use of enterotype to classify men with asthenozoospermia may help individualized treatment for different patients. For asthenozoospermia men with P enterotype, reducing their BMI may be helpful for their improvement of sperm quality.

Materials And Methods

Ethical statement

This study was conducted in accordance with the Code of Ethics and the 1975 Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Shengjing Hospital of China Medical University (Reference No. 2017PS190K). Informed consent was obtained from all participants.

Participants

All the participants recruited at Shengjing Hospital of China Medical University and Jinghua Hospital of Shenyang from October 2020 to April 2021. Inclusion criteria is age 18-49 years; patients who had taken antibiotics and probiotics within 1 month prior to the study were excluded. Blood sample was collected and measured on the day of clinic. Semen sample was obtained after abstain from ejaculation for 3–7 days. Fecal sample was taken and stored on the same day or the next day of semen collection. Sperm motility was divided into three categories: progressive, including rapidly and slowly progressive, non-progressive, and immotile according to the World Health Organization (WHO) criteria. The diagnostic criteria for asthenospermia are based on WHO laboratory manual for the examination and processing of human semen, and men who meet the criteria are diagnosed with asthenospermia, implying that the percentage of sperm forward movement in semen is less than 32%, and two or more semen analyses are recommended. Patients with obstructive and non-obstructive azoospermia were excluded. Ultimately, a total of 407 men were included in the study.

Parameter measurements

BMI was calculated by dividing body weight (kg) by height (m) squared. Levels of sex hormones—follicle-stimulating hormone (FSH), luteinizing hormone, estradiol, total testosterone (TT), and progestin—were measured with a chemiluminescence immunoassay. Semen samples were collected in a sterilized container through masturbation in a dedicated semen collection room; condoms or lubricants were not used. Semen analysis was conducted soon after liquefaction (<60 min). Sperm parameters including sperm concentration, total sperm count, and the percentage of each motility category of sperm were measured with WLJY9000, an instrument of computer-aided sperm analysis. Normal sperm reference values were determined according to WHO criteria. Throughout the study, external quality control was performed.

Extraction of microbiota DNA and quantitative polymerase chain reaction (qPCR) amplification

The fecal samples were stored at -20°C after collected and transported to the research center on dry ice within 24 hours of collection, where they were stored at -80°C until DNA was extracted. The TIANamp stool DNA kit (Tiangen Biotech [Beijing] Co., Ltd, Beijing, China) was used to extract the bacterial DNA from stool samples according to the manufacturer's protocol. DNA concentration was measured with a Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA). The concentration and purity of the recovered DNA fragments were determined via agarose gel electrophoresis and ultraviolet spectrophotometry. The OD260/OD280 ratio was set between 1.7–1.9. qPCR was performed with the Detect Genus Gut Microbes Detection Kit and PCR-Fluorescence Probe (Tiangen Biotech [Beijing] Co., Ltd). The *Prevotella* and *Bacteroides* primers were designed according to the results of the qPCR test (Supplementary Table 1). The amplification procedure was as follows: Samples were heated at 95 °C for five minutes, then shifted to 45 15 s cycles at 95 °C and one 40 s cycle at 56 °C. qPCR was performed on the ABI 7500 Real-Time PCR system (Applied Biosystems, Waltham, MA, USA), and the amplification conditions were set to achieve the best response.

Statistical methods

Enterotypes were identified by plotting the log-transformed abundance of *Bacteroides* versus the log-transformed abundance of *Prevotella*, which were calculated using the *diptest* package in R (The R Project for Statistical Computing, Vienna, Austria). A histogram plotting frequency of the log-transformed abundance of *Prevotella*/*Bacteroides* (P/B) was obtained using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). The Mann–Whitney *U* test of nonparametric coefficients was used to identify any significant differences in the clinical indices between the groups. The data in the tables were presented as median (interquartile range) and as histograms. Univariate and multivariate logistic regression analyses were carried out using SPSS statistics version 23.0 (IBM, Armonk, NY, USA). The receiver operating characteristic (ROC) curve was modeled using the GraphPad Prism 6 (GraphPad Software).

Declarations

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Jiao Jiao , Bochen Pan, Xuan Zhu and Xiuxia Wang made substantial contributions to conception or design of the work; Ze Xing, Sitong Dong, Gaoyu Li, Weifan Yao and Xinrui Yao made substantial contributions to the acquisition, analysis of the data; Jiao Jiao, Peng Xu and Xiaobin Wang made interpretation of data and drafted the work; Renhao Guo and Tao Feng substantively revised it.

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Data availability statement

The data that support the findings of this study are available in figshare at https://doi.org/10.6084/m9.figshare.19386542.v1, reference number 25.

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Tables

Table 1 Characteristics of participants' clinical parameters included in this study

		N=407
basic information, *N* (%)		
age	*Median (P25, P75)*	34.00(31.00-38.00)
	<40	349(85.7)
	≥40	58(14.3)
BMI	*Median (P25, P75)*	25.09(23.15-27.38)
	<24	135(33.2)
	≥24	272(66.8)
sperm quality, *N* (%)		
rate of forward progressive motility	*Median (P25, P75)*	28.84(20.54-41.46)
	Astheno (<32% motile)	234(57.5)
	normal	173(42.5)
rate of total motility	*Median (P25, P75)*	38.36(27.59-53.33)
	Astheno (<40% motile)	220(54.1)
	normal	187(45.9)
total sperm count	*Median (P25, P75)*	174.23(95.32-280.00)
	Low (<39 million)	11(2.7)
	normal	396(97.3)
sperm concentration	*Median (P25, P75)*	50.49(32.14-79.29)
	Oligo (<15 million/mL)	11(2.7)
	normal	396(97.3)
intestinal microbiome, *N* (%)		
P/B	*Median (P25, P75)*	0.39(0.00-3.68)
	<0.01	178(43.7)
	³0.01	229(56.3)
sex hormone, *M*(*P25,P75*)		
FSH	*Median (P25, P75)*	4.52(3.39-6.04)
LH	*Median (P25, P75)*	3.12(2.24-4.25)
E_2	*Median (P25, P75)*	33.79(25.51-41.40)
PRL	*Median (P25, P75)*	6.39(4.83-8.22)
TT	*Median (P25, P75)*	3.58(2.76-4.45)

Abbreviations: BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E_2, estradiol; TT, total testosterone; PRL, prolactin; P/B, *Prevotella/ Bacteroides*. Median (interquartile range) are shown. The Mann–Whitney U test of nonparametric coefficients was used for non-normally distributed data.

Table 2 Characteristics of the normal men and the overweight/obese men

		BMI		*P*
		normal (<24kg/m^2)	overweight/obese(≥24kg/m^2)	
		N=135	*N*=272	
basic information, *N* (%)				
age	<40	120(88.9)	229(84.2)	0.202
	≥40	15(11.1)	43(15.8)	
sperm quality, *N* (%)				
rate of forward progressive motility	Astheno (<32% motile)	64(47.4)	170(62.5)	**0.004**
	normal	71(52.6)	102(37.5)	
rate of total motility	Astheno (<40% motile)	57(42.2)	163(60.0)	**0.001**
	normal	78(57.8)	109(40.0)	
total sperm count	Low (<39 million)	4(3.0)	7(2.6)	0.758
	normal	131(97.0)	265(97.4)	
sperm concentration	Oligo (<15 million/mL)	4(3.0)	7(2.6)	0.758
	normal	131(97.0)	265(97.4)	
intestinal microbiome, *N* (%)				
P/B	<0.01	61(45.2)	117(43.0)	0.678
	≥0.01	74(54.8)	155(57.0)	
sex hormone, *M*(*P25,P75*)				
FSH		4.47(3.25-5.86)	4.56(3.44-6.09)	0.393
LH		3.17(2.35-4.36)	3.12(2.21-4.17)	0.462
E_2		34.24(24.39-42.48)	33.75(26.21-40.07)	0.777
PRL		6.44(4.65-8.07)	6.32(4.87-8.31)	0.976
TT		4.16(3.33-5.41)	3.27(2.60-4.16)	**<0.001**

Abbreviations: BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E_2, estradiol; TT, total testosterone; PRL, prolactin; P/B, *Prevotella*/ *Bacteroides*. Median (interquartile range) are shown. The Mann–Whitney U test of nonparametric coefficients was used for non-normally distributed data.

Table 3 Logistic regression analysis of risk factors for asthenospermia in all participants

	rate of forward progressive motility		*P*	rate of total motility			total sperm count	
BMI, *N*	<32% motile	normal		c	normal		<39 million	normal
<24	64	71	**0.004**	57	78	**0.001**	4	7
≥24	170	102		163	109		131	265
P/B, N								
<0.01	102	76	0.945	98	80	0.721	7	171
≥0.01	132	97		122	107		4	225
sex hormone								
FSH	4.70(3.41-6.39)	4.42(3.26-5.61)	**0.046**	4.71(3.48-6.32)	4.40(3.21-5.63)	**0.031**	7.56(5.52-9.82)	4.47(3.33-5.92)
LH	3.26(2.23-4.22)	3.03(2.24-4.27)	0.703	3.12(2.22-4.12)	3.14(2.31-4.36)	0.675	4.18(2.94-5.13)	3.11(2.23-4.19)
E_2	33.00(24.67-41.21)	35.05(26.85-41.76)	0.149	33.58(25.29-41.86)	34.86(25.82-40.81)	0.662	26.88(23.13-47.64)	34.04(25.67-41.36)
PRL	6.40(4.90-8.21)	6.29(4.69-8.24)	0.845	6.49(4.94-8.27)	6.24(4.60-8.18)	0.266	6.39(4.97-7.31)	6.39(4.81-8.25)
TT	3.54(2.68-4.41)	3.63(2.87-4.71)	0.385	3.48(2.66-4.37)	3.67(2.88-4.65)	0.13	4.18(3.26-5.62)	3.58(2.72-4.42)
curde OR	**rate of forward progressive motility**	***P***	**rate of total motility**	***P***	**total sperm count**	***P***	**sperm concentration**	***P***
BMI	1.849(1.218-2.807)	**0.004**	2.046(1.346-3.111)	**0.001**				
P/B								
sex hormone								
FSH	0.898(0.825-0.979)	**0.014**	0.885(0.812-0.964)	**0.005**	0.825(0.726-0.938)	**0.003**	0.785(0.681-0.904)	**0.001**
LH							0.759(0.609-0.946)	**0.014**
E_2								
PRL								
TT								
adjusted OR	**rate of forward progressive motility**	***P***	**rate of total motility**	***P***	**total sperm count**	***P***	**sperm concentration**	***P***
BMI	1.793(1.177-2.729)	**0.006**	1.981(1.299-3.022)	**0.002**				
P/B								
sex hormone								
FSH	0.903(0.828-0.985)	**0.022**	0.890(0.815-0.971)	**0.009**			0.801(0.674-0.952)	**0.012**
LH							0.944(0.705-1.265)	0.700
E_2								
PRL								
TT								

Abbreviations: BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E_2, estradiol; TT, total testosterone; PRL, prolactin; P/B, *Prevotella*/ *Bacteroides*. Median (interquartile range) are shown. The Mann–Whitney U test of nonparametric coefficients was used for non-normally distributed data.

Table 4 Logistic regression analysis of risk factors for asthenospermia in men with enterotype P

	rate of forward progressive motility		*P*	rate of total motility				total sperm count	*P*	sp
BMI, *N*	<32% motile	normal		<40% motile	normal		<39 million	normal		<15 million/mL
<24	28	46	**<0.001**	22	52	**<0.001**	1	73	1.000	3
≥24	104	51		100	55		3	152		4
sex hormone										
FSH	4.59(3.46-6.61)	4.09(3.14-5.64)	**0.036**	4.59(3.46-6.61)	4.09(3.14-5.64)	**0.005**	8.69(6.03-13.27)	4.37(3.27-6.04)	**0.009**	7.56(3.15-14.42)
LH	3.26(2.29-4.28)	3.09(2.45-4.27)	0.737	3.26(2.29-4.28)	3.09(2.45-4.27)	0.508	5.70(3.21-7.74)	3.17(2.30-4.26)	0.050	4.92(3.31-7.40)
E_2	33.69(25.49-42.47)	35.30(28.12-42.38)	0.285	33.69(25.49-42.47)	35.30(28.12-42.38)	0.841	30.45(22.21-45.92)	34.31(26.85-42.42)	0.578	32.76(28.1 34.86)
PRL	6.28(4.86-7.89)	6.52(4.67-8.25)	0.716	6.28(4.86-7.89)	6.52(4.67-8.25)	0.506	6.61(6.41-7.17)	6.29(4.71-8.18)	0.623	6.48(4.86-7.31)
TT	3.51(2.61-4.41)	3.81(3.00-4.91)	0.086	3.51(2.61-4.41)	3.81(3.00-4.91)	**0.001**	4.59(2.91-5.88)	3.58(2.85-4.50)	0.293	5.00(2.49-6.17)
curde OR	**rate of forward progressive motility**	***P***	**rate of total motility**	***P***	**total sperm count**	***P***	**sperm concentration**	***P***		
BMI	3.350(1.881-5.966)	**<0.001**	4.298(2.365-7.809)	**<0.001**						
P/B										
sex hormone										
FSH	0.869(0.772-0.977)	**0.019**	0.819(0.722-0.929)	**0.002**	0.848(0.732-0.982)	**0.027**				
LH							0.581(0.403-0.840)	**0.004**		
E_2										
PRL										
TT			1.300(1.072-1.577)	**0.008**						
adjusted OR	**rate of forward progressive motility**	***P***	**rate of total motility**	***P***	**total sperm count**	***P***	**sperm concentration**	***P***		
BMI	3.131(1.749-5.607)	**<0.001**	3.387(1.804-6.357)	**<0.001**						
P/B										
sex hormone										
FSH	0.886(0.785-1.002)	0.053	0.817(0.714-0.936)	**0.003**						
LH										
E_2										
PRL										
TT			1.212(0.988-1.487)	0.066						

Abbreviations: BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E_2, estradiol; TT, total testosterone; PRL, prolactin; P/B, *Prevotella*/ *Bacteroides*. Median (interquartile range) are shown. The Mann–Whitney U test of nonparametric coefficients was used for non-normally distributed data.

Figures

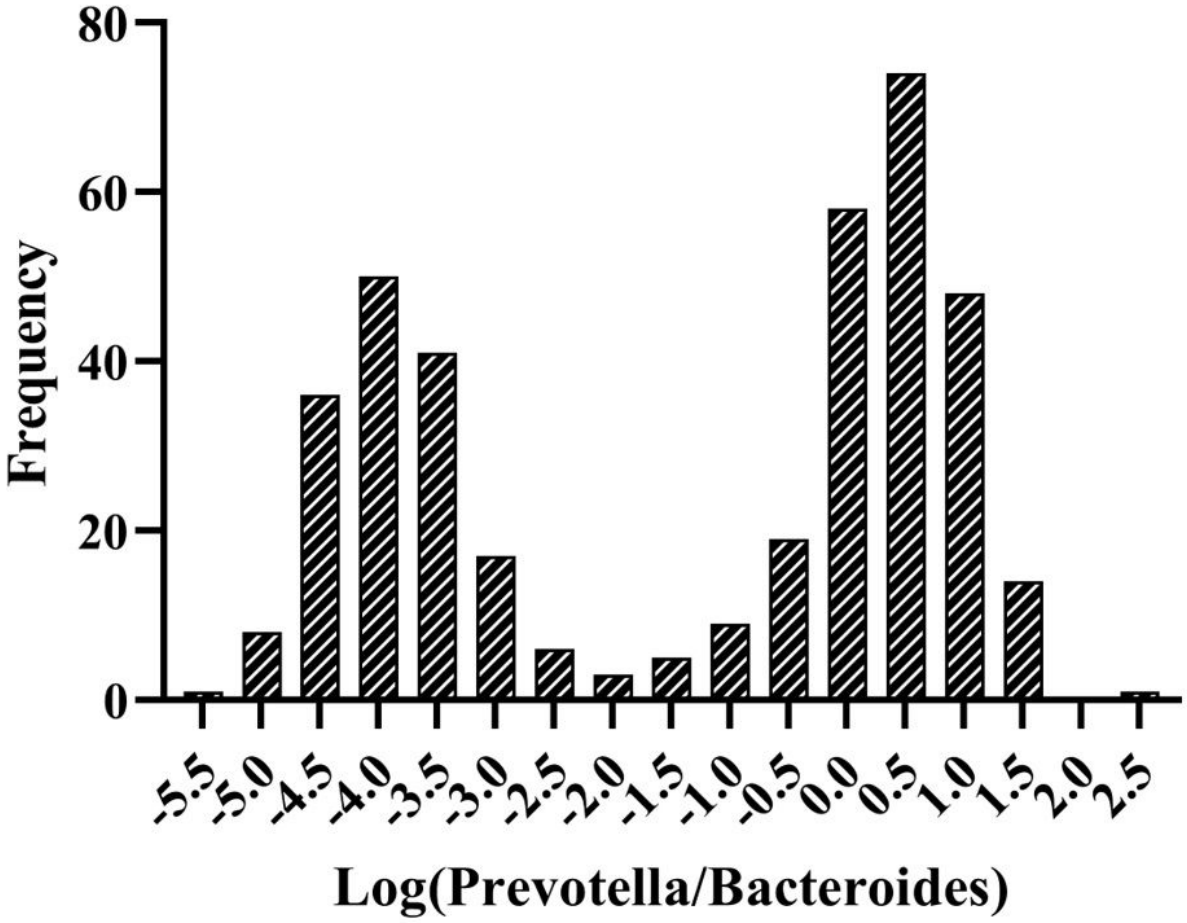


Figure 1

Prevotella/ *Bacteroides* (P/B) groups

Histogram plotting frequency of the log-transformed abundance of P/B for all patients.

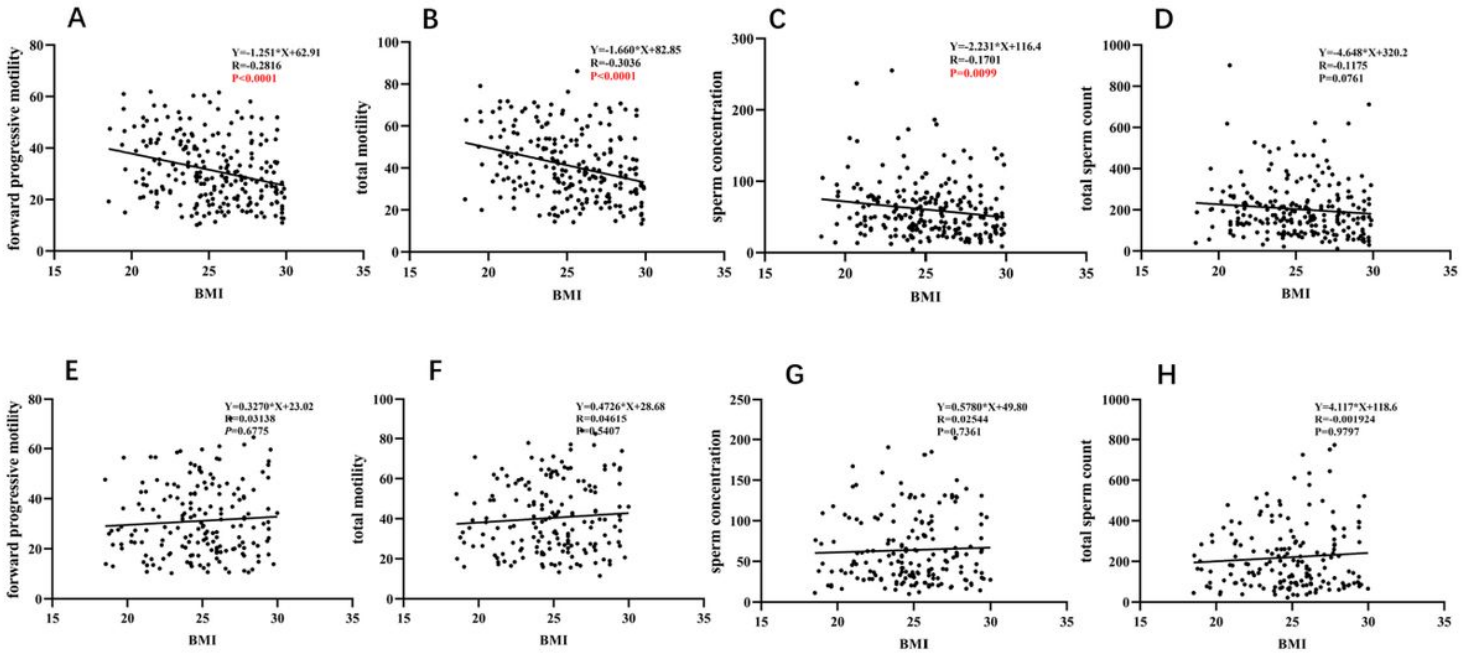
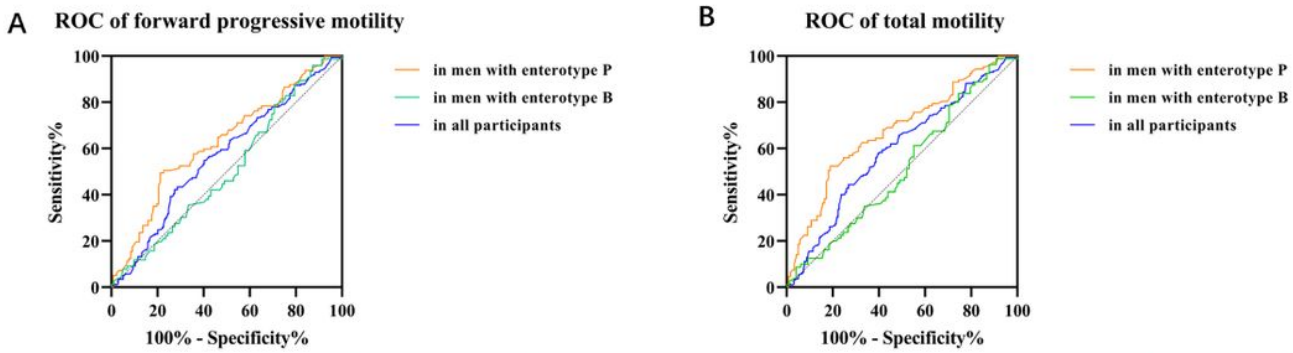


Figure 2

Correlation between BMI and sperm quality in men with enterotypes P and B

(A) Correlation between BMI and forward progressive sperm motility in men with enterotype P. (B) Correlation between BMI and total sperm motility in men with enterotype P. (C) Correlation between BMI and sperm concentration in men with enterotype P. (D) Correlation between BMI and total sperm count in men with enterotype P. (E) Correlation between BMI and forward progressive sperm motility in men with enterotype B. (F) Correlation between BMI and total motility in men with enterotype B. (G) Correlation between BMI and sperm concentration in men with enterotype B. (H) Correlation between BMI and total sperm count in men with enterotype B.

Abbreviations: BMI, body mass index.



		AUC±SE	95%CI	P value	Cut-off value	sensitivity	specificity
Forward progressive motility	In all participants	0.5673±0.0286	0.5112-0.6235	0.0201	24.1877	0.714	0.434
	In men with enterotype P	0.6273±0.0374	0.5539-0.7006	0.0010	24.0835	0.788	0.495
	In men with enterotype B	0.5095±0.0435	0.4241-0.5949	0.8289	20.6381	0.961	0.127
Total motility	In all participants	0.5927±0.0282	0.5374-0.6481	0.0013	24.9907	0.600	0.683
	In men with enterotype P	0.6753±0.0356	0.6055-0.7451	<0.0001	24.1877	0.811	0.523
	In men with enterotype B	0.5143±0.0434	0.4292-0.5995	0.7422	22.4277	0.838	0.255

Figure 3

Diagnostic performance of BMI with respect to asthenospermia incidence

(A) Diagnostic potential of enterotypes in predicting the incidence of decreased forward progressive sperm motility. **(B)** Diagnostic potential of enterotypes in predicting the incidence of decreased total sperm motility.

Abbreviations: AUC, area under curve; BMI, body mass index.

Supplementary Files

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- Supplementaltable.docx