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Seroprevalence of HSV-2 in multiple subgroups of infertile men with abnormal sperm parameters and those seeking sex selection: a case-control study

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Abstract

Background Herpes simplex virus type 2 (HSV-2) is a common sexually transmitted infection (STI) primarily acquired through sexual contact. In 2000, the World Health Organization (WHO) for the first time reported the association of STIs with male infertility. Infertility is described as the inability to achieve a clinical pregnancy after engaging in regular, unprotected sexual intercourse for a year or more. HSV-2 infection is a significant concern for infertility, with evidence suggesting it can contribute to a range of reproductive complications. The seroprevalence of HSV-2 among infertile men in Iran has not yet been determined. Therefore, we conducted a case-control study to examine the seroprevalence of HSV-2 in multiple subgroups of infertile men with abnormal sperm parameters (Case) and individuals seeking sex selection services and those with normal sperm parameters (Control).

Method Blood samples were collected from infertile males seeking fertility treatment, and those seeking sex selection at the Avicenna Infertility Clinic in Tehran, Iran between July 2023 and February 2024. Demographic and clinical data were collected through a questionnaire. Anti-HSV-2 IgG antibodies (Abs) were detected using a commercially available ELISA kit [Herpes simplex 2 (gG2 purified), Vircell, Spain].

Results Of the 486 samples that met the eligibility criteria, 420 were tested. The ELISA testing was performed on 98 control, 32 Teratozoospermia (T), 45 Asthenozoospermia (A), 48 Oligoteratozoospermia (OTA), 50 Azoospermia (Azo), and 147 Teratozooasthenospermia (TA) samples. Thirteen samples came out positive for HSV-2 IgG Abs (3.41% case and 2.04% control group). Of these 13 positive samples, 7 were from the TA group, 2 from the T group, 2 from the Azo group, and 2 control. The age (43.15 ± 5.10 vs. 37.74 ± 6.20, $p = 0{\cdot}0020$) and marriage duration (12.54 ± 6.88 vs. 8.12 ± 4.95, $p = 0{\cdot}0019$) were significantly higher in the HSV-2 IgG Ab positive group.

Conclusion The results of this study enhance our understanding of the epidemiology of HSV-2 in individuals seeking infertility treatment. It can be inferred that the seroprevalence of HSV-2 IgG among patients referring to an infertility treatment clinic in Tehran is relatively low. The study also indicates that the chance of HSV-2 infection increases with

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age. The highest number of positive cases in the TA suggests that HSV-2 may adversely affect sperm motility and morphology.

Keywords Herpes simplex virus type 2 (HSV-2), Infertility, Fertility, Genital infections, Sperm parameters, Seroprevalence

Introduction

Herpes simplex virus type 2 (HSV-2) is a common sexually transmitted infection (STI) primarily acquired through sexual contact and manifests as genital herpes [1]. HSV-2 belongs to the *Herpesviridae* family and contains a large, linear double-stranded DNA genome that replicates in the nuclei of host cells [2]. Infection with HSV-2 is lifelong and is marked by intermittent viral shedding from infected cells and the appearance of often painful sores or lesions in the genital area [1]. In 2012, there were 417 million individuals globally between the ages of 15 and 49 who had HSV-2 infection, which accounted for 11.3% of the population in that age group [3]. In 2000, the WHO for the first time reported the association of STIs with male infertility [4] bringing attention to the potential impact of STIs on male reproduction.

Infertility is described as the inability to achieve a clinical pregnancy after engaging in regular, unprotected sexual intercourse for a year or more [5]. It is estimated that 186 million individuals globally may be impacted by infertility, with male infertility accounting for over half of all cases [6]. Studies suggest that infertility affects about 8–12% of couples in the reproductive age group worldwide [6, 7]. Male infertility typically results from issues such as impaired sperm function, blockages that hinder sperm delivery, hormone imbalances, varicocele, malignancies, and infections [5]. HSV-2 infection is a significant concern for infertility, with evidence suggesting it can contribute to a range of reproductive complications [8]. Semen analysis (SA) represents the most basic evaluation of male infertility. According to the sixth edition of the WHO manual for human semen analysis, the assessment of semen parameters involves three key characteristics of sperm, namely, count, motility, and morphology [9]. The standard range for sperm count is 39 million per ejaculate, total motility is 42%, and normal morphology is 4%. Considering the presence of disorders within each sperm characteristic, abnormalities associated with sperm are categorized into various groups. The most prevalent of these include Azoospermia (Azo), Teratozoospermia (T), Asthenozoospermia (A), Teratozooasthenospermia (TA), and Oligoteratozoospermia (OTA) [10]. Azo is defined as the complete absence of sperm in a man's ejaculate. T is characterized by a high percentage of abnormally shaped sperm. A refers to reduced sperm motility. TA combines both teratozoospermia and asthenozoospermia, indicating that the sperm are both abnormally shaped and have reduced motility. OTA is characterized by a low sperm count (oligospermia) and abnormal sperm morphology [11]. STIs can impact male infertility through various mechanisms, with the primary one being the direct effect of the organism on semen quality, leading to spermatozoa apoptosis [12]. Prior studies have shown that HSV was associated with low sperm count and poor motility [13–15]. The seroprevalence of HSV-2 among infertile men in Iran has not yet been determined. Therefore, we conducted a case-control study to examine the seroprevalence of HSV-2 IgG antibody (Ab) in several subgroups of infertile men with abnormal sperm parameters (Case group) and individuals seeking sex selection services and those with normal sperm parameters (Control group) to determine their previous exposure.

Materials and methods

Study design, setting, and study population

In the present case-control study, the sample collection was conducted at the Avicenna Infertility Clinic in Tehran, Iran between July 2023 and February 2024. Infertile male patients seeking fertility treatment, as well as those seeking sex selection services, consented to participate and provide blood samples for serological investigation. To be eligible for participation in the case group, individuals had to meet the following criteria: (1) belong to one of the multiple subgroups of infertile men including Azoospermia (Azo), Teratozoospermia (T), Asthenozoospermia (A), Oligoteratozoospermia (OTA), and Teratozooasthenospermia (TA) seeking fertility treatment (2) aged ≥ 18 years (3) married for more than one year. Men seeking sex selection services (with normal sperm) and individuals with normal sperm parameters were considered as the control group. Individuals under 18 years old, those with varicocele or a history of mumps, people who had been married for less than a year, and samples that were hyperlipemic or exhibited hemolysis were excluded. Demographic and clinical data including age, length of marriage, infertility duration, history of genital infection, history of mumps, and medical conditions were collected through a questionnaire (Supplementary 1). Additionally, semen analysis (total count, motility, morphology, and white blood cell count) and infection status for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) were retrieved from the patient's medical records. Ethical approval of the study

was obtained from a medical ethics committee of Tarbiat Modares University (IR.MODARES.REC.1402.060).

Laboratory procedure & serological assay

Serum samples were obtained by centrifuging the blood specimens at 3000 rpm for 8 min. The separated serum samples were stored at -80 °C until the assay was performed. Every serum sample in the test met the required volume and was in good condition, free from hemolysis or lipemic specimens. Anti-HSV-2 IgG Abs were detected using a commercially available enzyme-linked immunosorbent assay (ELISA) kit [Herpes simplex 2 ELISA IgG/IgM (gG2 purified), Vircell, Spain] with a sensitivity of 91% and specificity of 100%. Microtiter plates of the Vircell HSV-2 IgG ELISA kit were coated with recombinant glycoprotein gG2. This allowed for the differentiation of type-specific antibody responses to HSV-2 infection by indirect ELISA. All tests were performed according to the manufacturer's instructions. Briefly, serum specimens were pipetted into the wells, where binding occurs between the class-specific IgG Abs in the serum and the immobilized herpes simplex antigen. After 45 minute (min) of incubation at 37 °C, the plate is rinsed with diluted wash solution, to remove unbound material. Then, ready-to-use antihuman (IgG) peroxidase conjugate is added and incubated for 30 min. After further washing, the substrate 3,3',5,5'-Tetramethylbenzidine (TMB) solution is pipetted and incubated for 20 min, inducing the development of a blue dye in the wells. The color development is terminated by adding a stop solution which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The antibody index (AI) was calculated by dividing the sample optical density (OD) by the cutoff value [AI= (sample OD/cut-off serum mean OD) x 10]. The IgG results were interpreted as follows: an AI < 0.5 was considered negative, an AI between 9 and 11 was considered borderline and an AI > 11 was considered positive. Following the Vircell kit protocol, borderline results were considered seronegative.

Statistical analysis

Qualitative variables were reported as frequency and percentage, and quantitative variables were reported as mean ± standard deviation (SD). The chi-square or Fisher exact test was used to analyze categorical variables. Student t-test was used to compare quantitative variables between two groups. A one-way ANOVA test was used to compare quantitative variables among more than two groups. Shapiro-Wilk test was used to evaluate the normality of continuous variables. The R version 4.4.1 was used for all statistical analyses with p-values < 0.05 defined as statistically significant.

Results

Samples' characteristics

During the study period, a total of 532 serum samples were collected. Of these, 150 samples were obtained from individuals seeking sex selection and those with normal semen parameters and designated as the control group. The remaining 382 samples were categorized based on the analysis of sperm parameters as follows: 37 samples were classified as teratospermia (T), 48 as asthenospermia (A), 58 as azoospermia (Azo), 61 as Oligoteratoasthenospermia (OTA), and 178 as teratoasthenospermia (TA). The samples were screened based on the established inclusion/exclusion criteria, and 46 samples (23 due to a history of mumps, 8 with an infertility duration < 1, 7 with a marriage duration < 1, 6 with varicocele, and 2 with hemolysis) were subsequently excluded. Ultimately, 486 samples of infertile men and the control group that met the eligibility criteria were categorized into the following subgroups: 37 T, 46 A, 54 Azo, 55 OTA, 160 TA, and 134 control groups.

Demographic and clinical characteristics of the participants

Among 486 samples, the mean ± standard deviation (Mean ± SD) of the age of the subjects was 37.9 ± 6.2 years. The age distribution was as follows: 35 (7.2%) were in the 20–29 age group, 258 (53%) were in the 30–39 age group, 174 (36%) were in the 40–49 age group, and 19 (3.9%) were 50 years of age and older. The mean ± SD of the marriage duration was 8.3 ± 5.1 years. A history of genital infection was reported in 6 people (1.2%). Among the patients who reported their comorbidities, the most common one was diabetes mellitus (2.3%). This was followed by hypertension, convulsion (1% each), hyperthyroidism (0.8%), ulcerative colitis, HBV, and favism (0.4% each), as well as acute lymphoblastic leukemia (ALL), asthma, Behçet's syndrome, and fatty liver (0.2% each). The serological status of hepatitis B surface antigen (HBS-Ag), hepatitis B surface antibody (HBS-Ab), hepatitis C virus antibody (HCV-Ab), and human immunodeficiency virus antibody (HIV-Ab) in 486 samples is presented in Table 1.

The results of the semen parameters analysis

The results of the semen parameters in 486 samples of infertile men and those seeking sex selection were 130 ± 110 for total sperm count ($\times 10^6$), 53 ± 35 for sperm count/ml ($\times 10^6$), 23 ± 13 (%) for motility and 253 ± 645 for WBC/ml ($\times 10^3$). Figures 1, 2, 3 and 4 outline the box plots of sperm parameters in different subgroups.

Seroprevalence of HSV-2 IgG antibodies

Of the 486 samples that met the eligibility criteria, 420 were tested using the Herpes simplex 2 ELISA IgG/IgM

Table 1 Serological status of HBS-Ag, HBS-Ab, HCV-Ab, and HIV-Ab in 486 samples of infertile men and the control group

Variable	Number
HCV-Ab	
Negative	385
Unknown	101
HBS-Ag	
Positive	1
Unknown	101
HBS-Ab	
Positive	176
Unknown	303
HIV-Ab	
Negative	385
Unknown	101

(gG2 purified) kit. The ELISA testing was performed on 98 control samples, 32 T samples, 45 A samples, 48 OTA samples, 50 Azo samples, and 147 TA samples. Thirteen samples (3.1%) came out positive for HSV-2 IgG Abs, and three samples (0.7%) were reported in the borderline range. Of these 13 positive samples, 11 (3.41%) were in the case group and 2 (2.04%) were in the control group. Of these 13 positive samples, 7 were from the TA group, 2 from the T group, 2 from the Azo group, and 2 control. The characteristics of the positive samples are listed in Table 2.

Comparison of demographic and clinical characteristics based on different studied groups

Table 3 presents a comparison of the demographic and clinical characteristics among the multiple subgroups of infertile men and the control group. According to Table 3, there was no significant difference in age ($p=0.25$) and marriage duration ($p=0{\cdot}055$) between the various subgroups of infertile men and the control group. In the control group, more than half of the individuals (55%) were between 30 and 39 years of age. In the other infertile subgroups, the majority of people were also in the 30–39 age range. Table 4 compares the demographic and clinical characteristics between the infertile and the control group. The total sperm count was significantly lower in the infertile group compared to the control group (99.16±89.90 vs. 210.08±117.17 million, respectively; $p<0{\cdot}0001$). Sperm motility in the control group was 35.93±5.64 and was significantly lower in the infertile group (17.60±11.33, $p<0{\cdot}0001$). A significant difference was seen between the two studied groups in the sperm count/ml ($p<0{\cdot}0001$). Table 5 compares the demographic and clinical characteristics of people who are positive for the presence of HSV-2 IgG Abs and those who are negative. The frequency of HSV-2 IgG Abs was not significantly associated with sperm parameters. The age (43.15±5.10 vs. 37.74±6.20, $p=0{\cdot}0020$) and marriage duration (12.54±6.88 vs. 8.12±4.95, $p=0{\cdot}0019$) were significantly higher in the HSV-2 IgG Ab positive group.

Discussion

The present study investigated the seroprevalence of previous exposure to HSV-2 among several subgroups of infertile men with abnormal sperm parameters, as well as individuals with normal sperm parameters and seeking sex selection in Tehran, Iran. The selected ELISA kit employed the HSV-2-specific glycoprotein G2 for the detection of HSV-2 IgG Abs with 100% specificity. The results revealed that 13 out of 420 participants (3.1%) had been exposed to HSV-2 by testing seropositive to HSV- 2 IgG Abs. Only two characteristics, age and marriage duration, were significantly associated with a higher prevalence of HSV-2 IgG Abs (Table 5). Herein, higher HSV-2 IgG Ab levels were observed in males with abnormal semen parameters, particularly in terms of abnormal morphology and low motility. Previous studies have proposed an association between HSV-2 and lower semen count. In the studies from Iran, positive HSV-2 in the semen of infertile men was correlated with lower sperm count [13] and abnormal morphology [16]. However, in the study by Neofytou et al., no association was found between the presence of viral DNA and semen parameters [17]. Kapranos, et al. (2003) investigated the prevalence of HSV, cytomegalovirus (CMV), and Epstein-Barr virus (EBV) in the semen of men with fertility problems. The study included 113 men attending an infertility clinic in Athens. The findings indicated that viral DNA was detected in 56.6% of semen samples, with HSV DNA being the most prevalent at 49.5%, followed by EBV at 16.8% and CMV at 7.1%. HSV was significantly associated with low sperm count and poor motility. In contrast, CMV and EBV did not show any correlation with sperm concentration and motility [14].

In addition, two key studies using transgenic animal models have provided experimental evidence for the link between HSV infection and male infertility. Huttner et al. found that expression of the HSV thymidine kinase (TK) gene in the testes of transgenic mice was associated with increased apoptosis of germ cells and structural and functional abnormalities in sperm [18]. The study in transgenic rats further corroborates the evidence from transgenic mice that HSV infection can cause germ cell apoptosis, disrupt spermatogenesis, and impair sperm quality, ultimately leading to reduced fertility [19].

The study conducted in the United Arab Emirates (UAE) by Abdo et al., in 2022 is one of the few that have investigated the seroprevalence of HSV-2 IgG Ab among patients seeking fertility treatment. This cross-sectional study in a specific population group found that 12.4% of the patients tested positive and the prevalence was higher in males (15.6%) compared to females (12.0%).

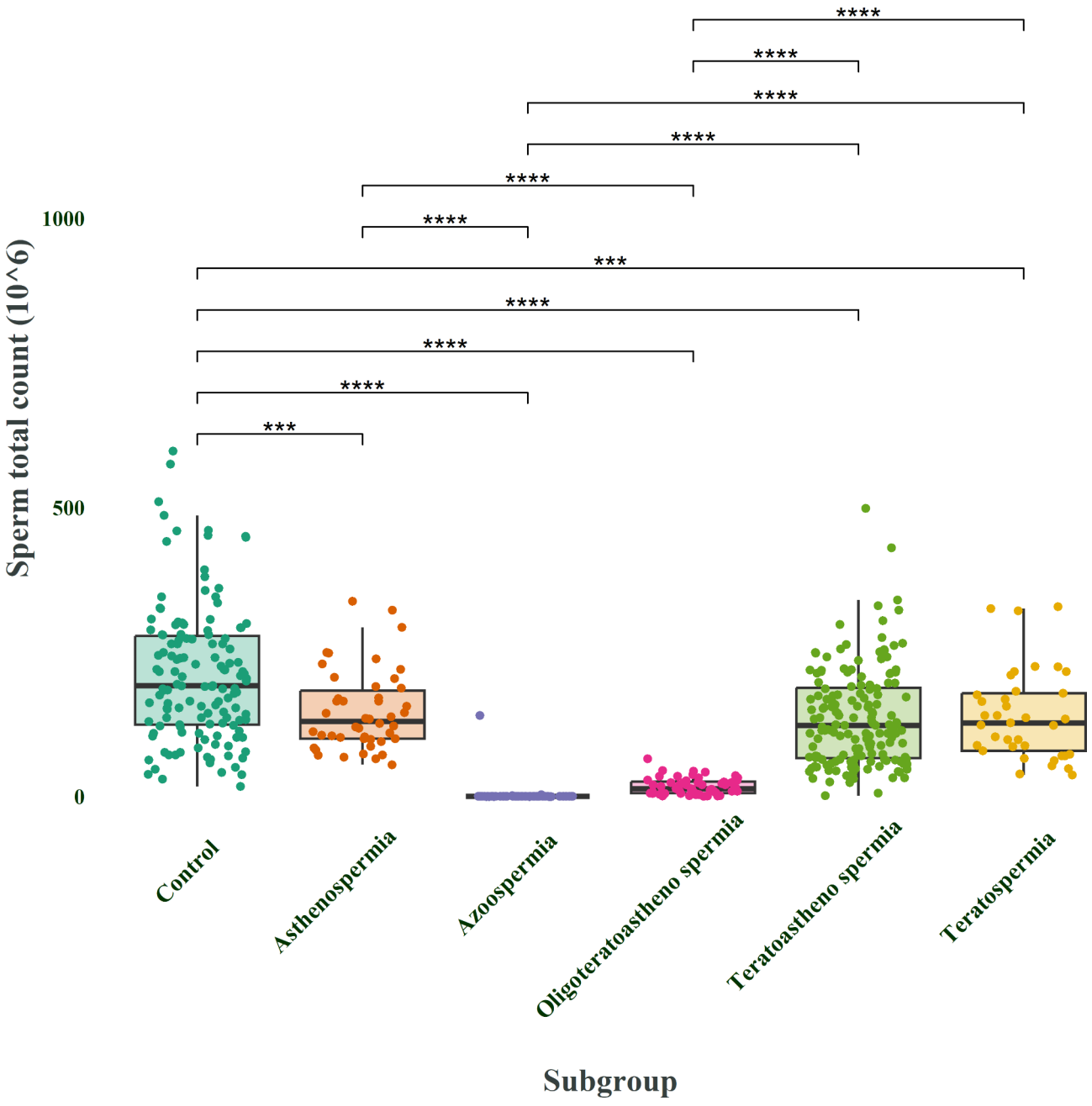

Fig. 1 Total sperm count in the semen samples of different subgroups of infertile men and the control group (**P* value < 0.05, ***P* value < 0.01,****P* value < 0.001, ,*****P* value < 0.0001)

Additionally, the study found that 69.0% of infertile males had abnormal semen analysis. The study also noted that patients with HSV-2 IgG seropositivity had a higher mean age (39.5 years) compared to seronegative patients (35.4 years) and that HSV-2 IgG seropositivity was more common in males with abnormal semen (10.3%) compared to those with normal semen (7.7%) [20]. It should be noted that the seroprevalence of HSV-2 among infertile individuals in the current study (3.1%) is substantially lower than the 12% reported in the 2022 UAE study. Several factors may contribute to this observed difference. The UAE has a diverse population comprising various ethnicities and nationalities, which may influence sexual behaviors and, consequently, the prevalence of HSV-2 infection.

Consistent with the findings of the aforementioned study, the highest HSV-2 seroprevalence was observed in the 40–50 years' age group (Table 2). This observation suggests that the prevalence of HSV-2 Abs increases with advancing age. This pattern can be attributed to the fact that the risk of HSV-2 infection is typically higher

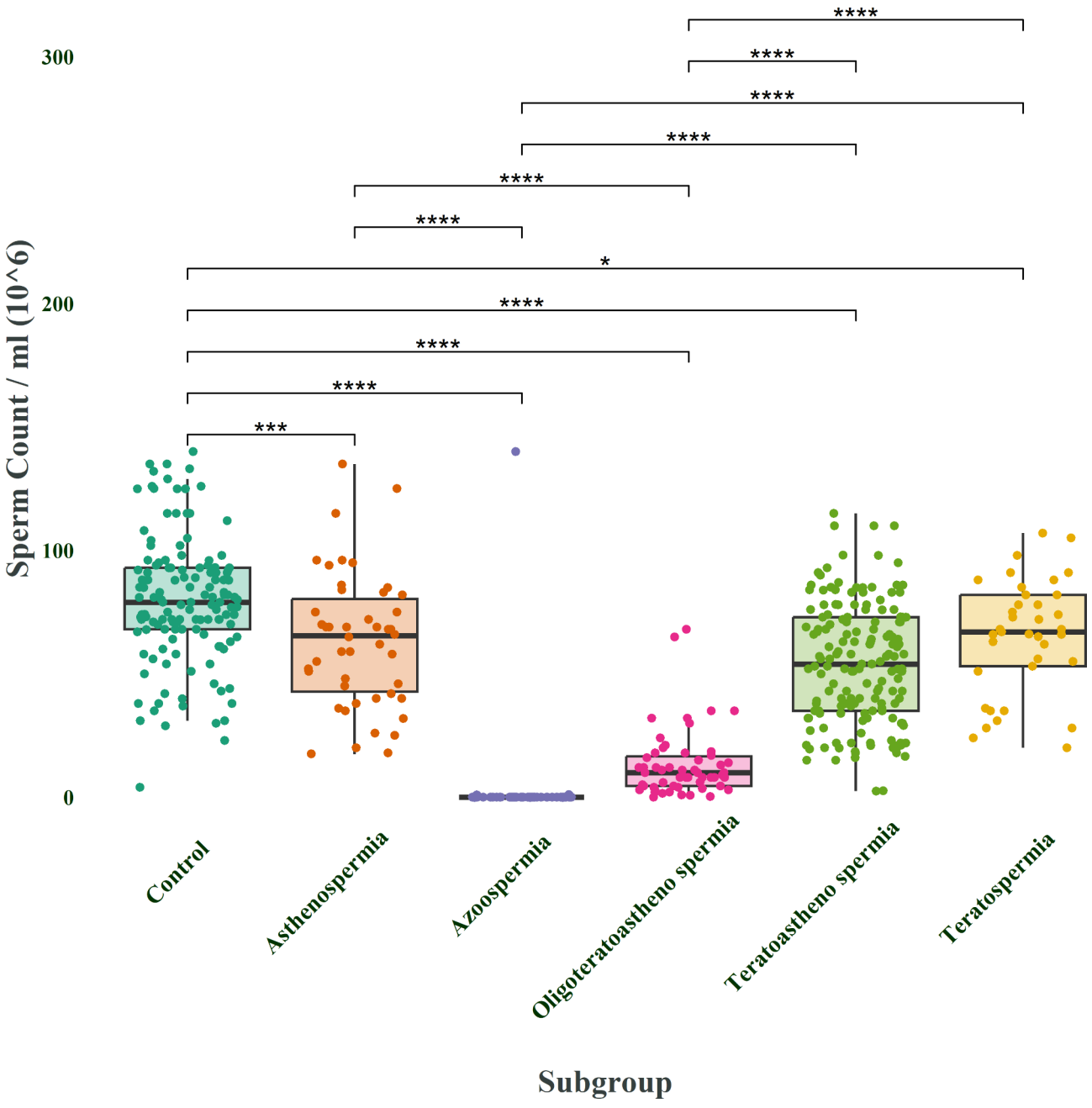

Fig. 2 Sperm count/ml of semen samples in different subgroups of infertile men and control group (**P* value < 0.05, ***P* value < 0.01, ****P* value < 0.001, ,*****P* value < 0.0001)

among individuals who have had more opportunities for exposure and transmission over time. Older individuals are more likely to have had multiple sexual partners and longer durations of sexual activity, which can contribute to a higher cumulative risk of contracting HSV-2. In a study conducted in Poland, Smith et al., reported that the prevalence of HSV-2 increased with age, with a threefold increase starting at age 40 (from 4% at age 15–24 to 12% at age 50–65 years) [21].

The seroprevalence of HSV-2 varies across different regions and countries. For example, the prevalence in the United States is reported to be around 15.7% [22], while it is notably lower in Jordan (2.9%) [23]. This disparity in HSV-2 seroprevalence underscores the influence of geographic and socioeconomic factors on the frequency of HSV infections.

The existing literature on HSV-2 seroprevalence in Iran has largely been conducted on the general population or high-risk groups, rather than the infertile population. A

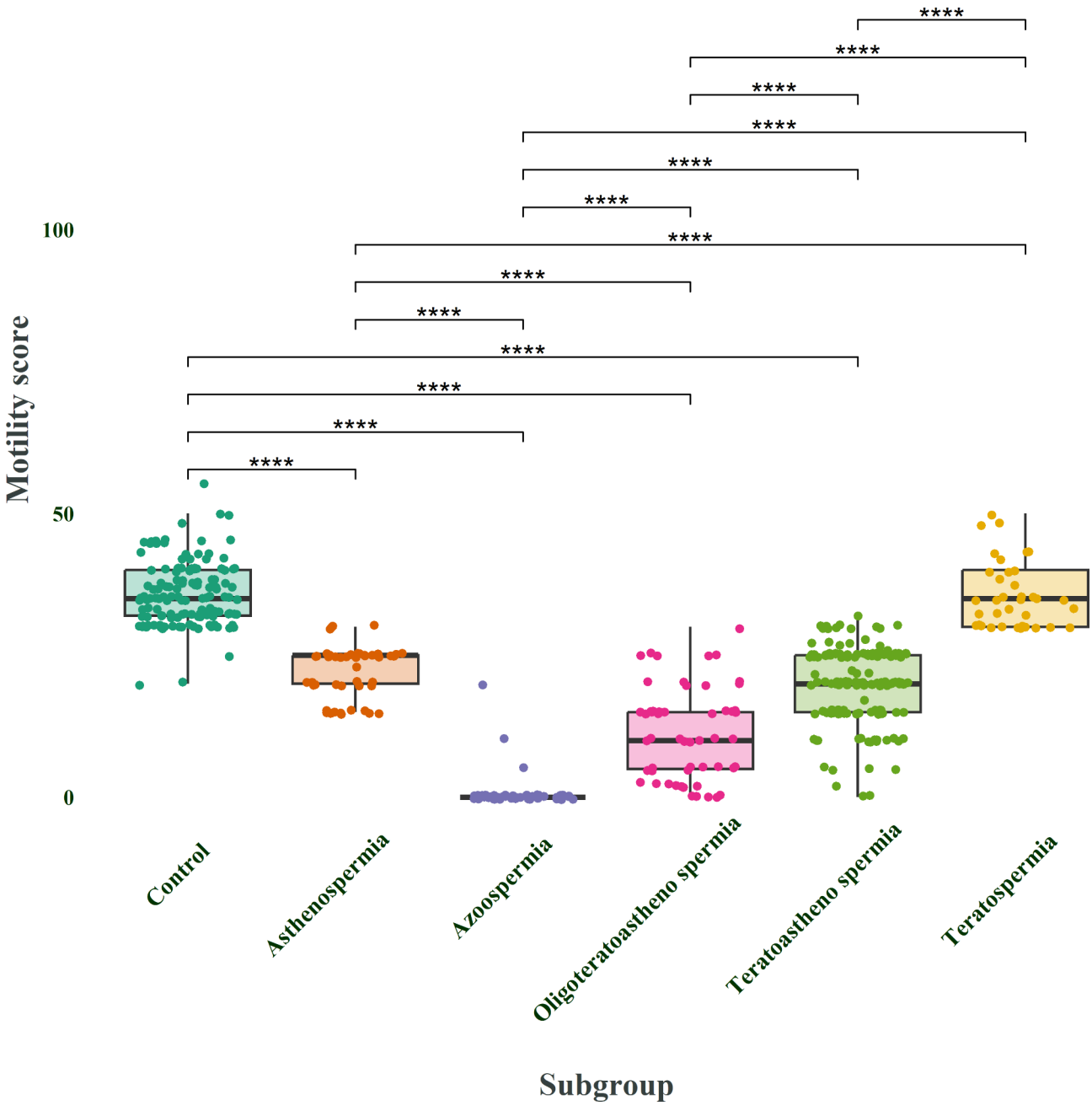

Fig. 3 Sperm motility in semen samples of different subgroups of infertile men and control group (**P* value < 0.05, ***P* value < 0.01,****P* value < 0.001, ,*****P* value < 0.0001)

study conducted in northern Iran in 2012 by Rezaei et al. investigated the seroprevalence of HSV-2 in the general population across various cities in Guilan Province. The study examined 800 randomly serum samples from individuals aged 1 to 85 years, revealing that 28 participants (3.5%) tested positive for the virus. Notably, the highest number of positive cases was reported among individuals over 40 years of age [24].

These findings underscore the importance of HSV-2 screening and education efforts, particularly among sexually active adults in the 30–40 years age range, to facilitate early detection, management, and prevention of HSV-2 transmission within this population.

Conclusion

Overall, the results of this study enhance our understanding of the epidemiology of HSV-2 in individuals seeking infertility treatment. It can be inferred that the seroprevalence of HSV-2 IgG among patients referring to an infertility treatment clinic in Tehran is relatively low. The

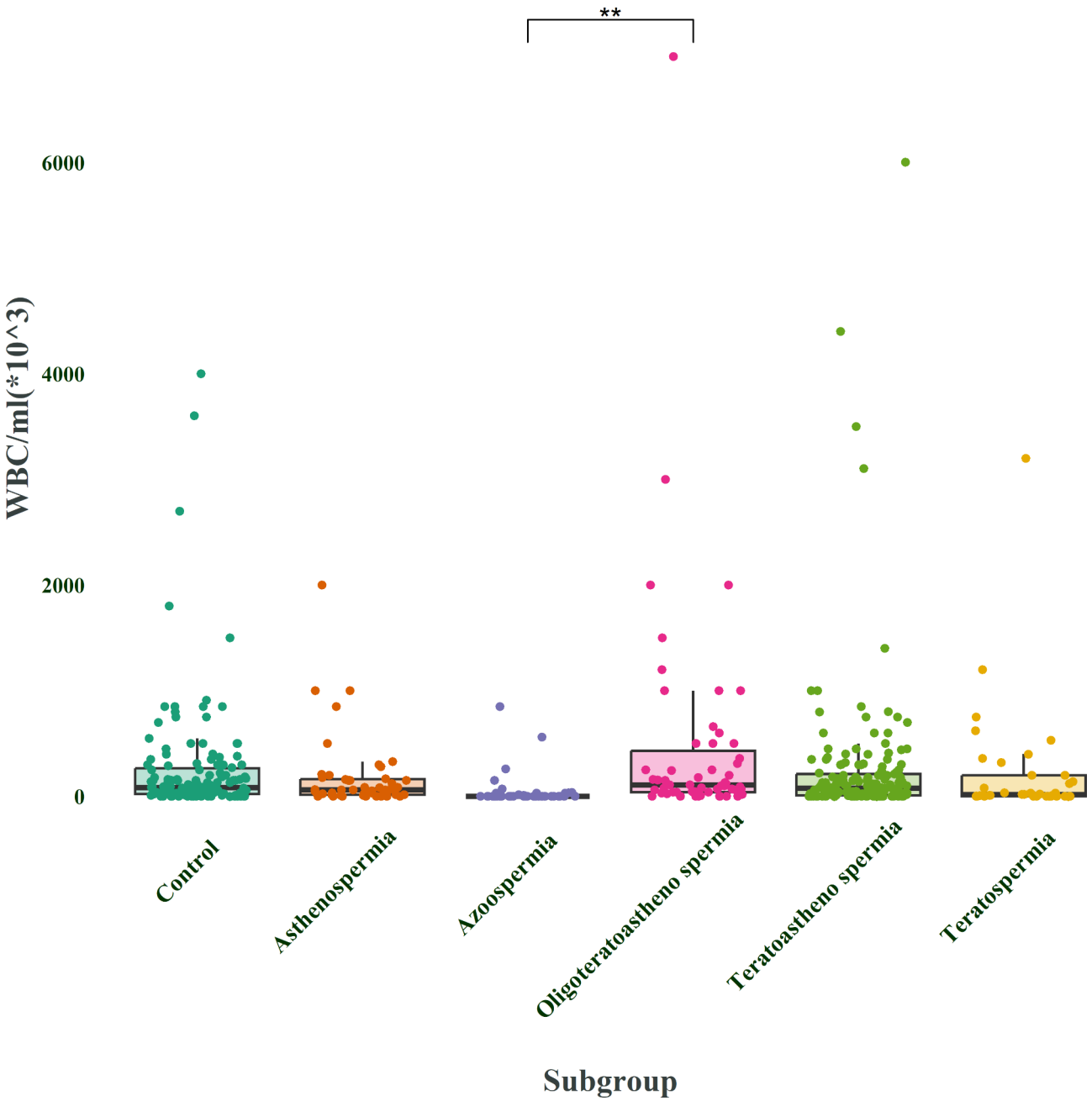

Fig. 4 Number of white blood cells (WBC)/ml of semen samples in different subgroups of infertile men and the control group (**P* value < 0.05, ***P* value < 0.01,****P* value < 0.001, ,*****P* value < 0.0001)

study also indicates that the chance of HSV-2 infection increases with age. Notably, the highest number of positive cases was observed among individuals with abnormal sperm analysis, particularly in the TA (7 out of 13 positive cases). This suggests that HSV-2 may adversely affect sperm motility and morphology.

Table 2 Characteristics of positive samples for HSV-2 IgG antibodies in 420 studied infertile men and control

Disease Type	Age (Year)	Marriage Duration (Year)	Genital Infection History	Sperm Total Count (× 106)	Sperm Count/ml (× 106)	Motility (%)	Infertility duration
Azo	46	15	No	0.00	0.0	0	15
Azo	53	21	No	0.00	0.0	0	21
Control	48	4	Yes	50.40	126.0	30	4
Control	43	20	No	72.00	72.0	37	20
TA	34	2	No	241.00	71.0	25	1
TA	41	6	No	249.00	83.0	20	1
TA	38	10	No	31.00	31.0	25	10
TA	42	16	No	215.00	43.0	27	11
TA	42	20	No	5.72	2.6	15	20
TA	49	22	No	178.00	78.0	25	22
TA	38	8	No	82.50	16.5	5	1
T	42	11	No	325.00	65.0	30	11
T	45	8	No	70.00	35.0	40	1

Table 3 Demographic and clinical characteristics among the multiple subgroups of infertile men and the control group

Variable	*N*	Subgroup						*p*-value[2]
		Control, *N* = 134 *1*	A, *N* = 46 *1*	Azo,*N* = 54 *1*	OTA, *N* = 55 *1*	TA, *N* = 157 *1*	T, *N* = 37 *1*	
Age	483	37·84 (5·40)	38·07 (6·42)	36·52 (7·49)	37·71 (6·29)	38·66 (6·25)	36·70 (5·39)	0·25
Marriage duration	483	7·96 (4·59)	9·28 (5·17)	7·87 (5·60)	7·96 (5·33)	8·92 (5·28)	6·35 (3·82)	0·055
Genital infection History	483	4 (3·0%)	0 (0%)	0 (0%)	0 (0%)	2 (1·3%)	0 (0%)	0·63
Sperm Total Count	483	210·08 (117·17)	147·45 (68·97)	2·68 (19·05)	17·52 (14·15)	137·23 (83·34)	139·78 (78·12)	**< 0·0001**
Sperm Count/ml	483	79·70 (25·68)	63·38 (27·21)	2·63 (19·05)	13·20 (13·75)	54·18 (24·49)	65·14 (23·14)	**< 0·0001**
Motility	482	35·93 (5·64)	22·13 (4·66)	0·65 (3·08)	11·30 (8·15)	20·05 (6·64)	35·51 (5·81)	**< 0·0001**
WBC	483	263·60 (564·33)	189·35 (362·39)	38·43 (141·21)	477·45 (1 074·81)	261·94 (708·51)	225·22 (566·38)	**0·025**
HSV2 Ab	420							0·30
Negative		96 (98%)	45 (100%)	48 (96%)	48 (100%)	140 (95%)	30 (94%)	
Positive		2 (2·0%)	0 (0%)	2 (4·0%)	0 (0%)	7 (4·8%)	2 (6·3%)	
HBS Ag	382	0 (0%)	0 (0%)	1 (2·1%)	0 (0%)	0 (0%)	0 (0%)	0·42
HBS Ab	181	37 (95%)	17 (94%)	21 (91%)	16 (89%)	70 (100%)	13 (100%)	**0·049**
Age groups	483							
20–29		7 (5·2%)	3 (6·5%)	9 (17%)	4 (7·3%)	9 (5·7%)	2 (5·4%)	
30–39		74 (55%)	23 (50%)	29 (54%)	33 (60%)	74 (47%)	25 (68%)	
40–49		49 (37%)	17 (37%)	12 (22%)	15 (27%)	71 (45%)	9 (24%)	
50 and more		4 (3·0%)	3 (6·5%)	4 (7·4%)	3 (5·5%)	3 (1·9%)	1 (2·7%)	

1 Mean (SD); n (%)

2 One-way ANOVA; Fisher's exact test

Table 4 Demographic and clinical characteristics between the infertile and the control group

Variable	N	Subgroup		p-value[2]
		Control, N = 134[1]	Infertile, N = 349[1]	
Age	483	37·84 (5·40)	37·89 (6·42)	0·92
Marriage duration	483	7·96 (4·59)	8·38 (5·23)	0·38
Genital infection History	483	4 (3·0%)	2 (0·6%)	0·053
Sperm Total Count	483	210·08 (117·17)	99·16 (89·90)	**< 0·0001**
Sperm Count/ml	483	79·70 (25·68)	42·12 (32·56)	**< 0·0001**
Motility	482	35·93 (5·64)	17·60 (11·33)	**< 0·0001**
WBC	483	263·60 (564·33)	247·86 (687·91)	0·80
HSV2_Ab	420			0·74
Negative		96 (98%)	311 (97%)	
Positive		2 (2·0%)	11 (3·4%)	
HBS Ag	382	0 (0%)	1 (0·3%)	> 0·99
HBS Ab	181	37 (95%)	137 (96%)	0·64
Age groups	483			0·79
20–29		7 (5·2%)	27 (7·7%)	
30–39		74 (55%)	184 (53%)	
40–49		49 (37%)	124 (36%)	
50 and more		4 (3·0%)	14 (4·0%)	

1 Mean (SD); n (%)

2 Welch Two Sample t-test; Fisher's exact test

Table 5 Demographic and clinical characteristics of people who are positive for the presence of HSV-2 IgG antibodies and those who are negative

Variable	N	HSV2 Ab		p-value[2]
		Negative, N = 407[1]	Positive, N = 13[1]	
Age	420	37·74 (6·20)	43·15 (5·10)	**0·0020**
Marriage duration	420	8·12 (4·95)	12·54 (6·88)	**0·0019**
Genital infection History	420	4 (1·0%)	1 (7·7%)	0·15
Sperm Total Count	420	130·07 (111·52)	116·89 (110·46)	0·67
Count/ml	420	51·37 (35·13)	47·93 (38·46)	0·73
Motility	419	21·92 (12·83)	21·46 (13·02)	0·90
WBC	420	246·26 (675·64)	210·77 (339·93)	0·85
Fertility type	420			0·30
Control		96 (24%)	2 (15%)	
Asthenospermia		45 (11%)	0 (0%)	
Azoospermia		48 (12%)	2 (15%)	
Oligoteratoastheno spermia		48 (12%)	0 (0%)	
Teratoastheno spermia		140 (34%)	7 (54%)	
Teratospermia		30 (7·4%)	2 (15%)	
HBS Ag	380	1 (0·3%)	0 (0%)	> 0·99
HBS Ab	181	169 (96%)	5 (100%)	> 0·99
Age groups	420			**0·037**
20–29		27 (6·6%)	0 (0%)	
30–39		226 (56%)	3 (23%)	
40–49		137 (34%)	9 (69%)	
50 and more		17 (4·2%)	1 (7·7%)	

1 Mean (SD); n (%)

2 One-way ANOVA; Fisher's exact test

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-024-10421-0.

Supplementary Material 1

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Author contributions
SSM designed and supervised the work. MJM collected the samples and performed the laboratory tests. SSM wrote the manuscript. HS contributed to sample collection.

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Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval consent to participate
Ethical approval of the study was obtained from the medical ethics committee of Tarbiat Modares Univesity (IR.MODARES.REC.1402.060). All methods were performed in accordance with the relevant guidelines and regulations. Informed consent was obtained from all individual participants included in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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