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Prevalence of *Neisseria Gonorrhoeae* Urogenital Tract Infections Among Patients with Infertility in Nablus, Palestine: Comparative Cross-Sectional Study

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Abstract: This study aimed to determine the prevalence of *Neisseria gonorrhoeae* urogenital tract infections among patients attending a gynecology clinic and infertility centers in Nablus city in Palestine. Vaginal swabs and semen specimens were collected from 238 patients attending gynecology and infertility centers in Nablus city in Palestine. *N. gonorrhoeae* presence was examined in all specimens by polymerase chain reaction (PCR) and in 163 (68.5%) specimens by selective GC agar culture. Among the examined specimens, PCR was positive in 2 specimens, while GC culture was negative in all 163 examined specimens, including the 2 PCR-positive cases. The prevalence of *N. gonorrhoeae* among the fertile group (1.1%) was close to that of the infertile group (0.7%). The history of urinary tract infections (UTIs) among the infertile group (61.1%) was significantly (P=0.000) higher than that (36%) of the fertile group. 97.8% of the infertile males had never used condoms, which was significantly (P=0.001) higher than that (53.3%) among fertile ones. The frequency of use of the seat toilet by infertile individuals (99.3%) was significantly (0.003) higher than that of fertile individuals. The study revealed that PCR was more sensitive than culture in detecting *N. gonorrhoeae* in clinical samples. There was no significant association between the infertility status and the presence of *N. gonorrhoeae* infection. Significant association was found between infertility and a number of variables such as history of UTIs, never using condoms, and frequency of using a seated toilet.

Keywords: *Neisseria gonorrhoeae*, infertility, PCR detection, GC culture, sexually transmitted infections (STIs), health sustainability, Nablus, Palestine.

Introduction

More than 186 million people are infertile, and most of them live in developing countries. About 1 out of 7 couples in the Western world and 1 out of 4 couples in the developing world have infertility (1). Infertility is the inability of a couple to conceive after a year of regular unprotected intercourse (2). Infertility can be primary or secondary. Primary infertility is diagnosed when the criteria for infertility are met in someone who has never conceived a child in the past, while secondary infertility is the diagnosis when the criteria are met with a previous history of achieving a successful pregnancy (1).

There are several causes of infertility in females and males. In females, infertility is caused by ovulatory disorders (polycystic ovarian syndrome), hyperprolactinemia, hypothalamic hypogonadism, premature ovarian insufficiency, hypothyroidism and congenital adrenal hyperplasia, fallopian tubal diseases (obstruction and pelvic adhesive disease), uterine causes (fibroids, polyps, intrauterine adhesions, Mullerian anomalies and cervical stenosis), oocyte abnormality like age- related aneuploidy and sexual transmitted infections (3, 4, 5).

Male factors of infertility can be divided into obstructive diseases (vasectomy, absence of the vas deferens and ejaculatory duct obstruction), nonobstructive diseases (Kallmann syndrome or other hypogonadotropic hypogonadism, testicular failure idiopathic or secondary to gonadal dysgenesis), hyperprolactinemia and exogenous testosterone exposure, functional disorders like erectile dysfunction and sexual transmitted infections (3, 4, 5).

Among females and males, sexually transmitted infections are considered one of the causes of infertility around the world (4, 5). Gonorrhoea is a common sexually transmitted infection (STI) worldwide. The causative agent of Gonorrhoea is Neisseria gonorrhoeae (*N. gonorrhoeae*), which is a Gram-negative diplococcus bacterium capable of infecting the urogenital, rectal, and pharyngeal sites (6, 7).

While males and females can both experience symptoms, the majority of cases of gonorrhea are asymptomatic (8). Usually, Gonorrhoea is more often symptomatic in males than

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females. Males suffer from urethral discharge accompanied by itching and dysuria, testicular pain and/or swelling, and rectal pain. When it's symptomatic in females it presents as vaginal discharge accompanied with dysuria, abnormal vaginal bleeding, lower abdominal and rectal pain, and dyspareunia (6).

The pooled 2016 global prevalence of urogenital gonorrhoea (the proportion of the world's population with gonorrhoea in a given year) was estimated by the World Health Organization (WHO) to be 0.9% in women and 0.7% in men, corresponding to a total of 30.6 million gonorrhoea cases worldwide and not just that what made gonorrhoea a major cost burden on the health care system, but also the serious complications that this disease can cause (9).

In females, untreated *N. gonorrhoeae* infections of the genital tract may lead to complications such as ectopic pregnancy, pelvic inflammatory disease, and infertility (10). In some cases, in both genders, *N. gonorrhoeae* may penetrate the circulation and disseminate, leading to cutaneous and tendon or joint infections and, rarely, endocarditis or meningitis (11).

Research gap

Sexually transmitted infections (STIs) have been one of the important global health challenges, and are considered one of the major causes of patients' morbidity around the world. Thus, they are a major cost burden on both the patients themselves and the government (12). Moreover, the lack of data about the association between STIs and infertility in Palestine specifically motivated us to study the prevalence of *N. gonorrhoeae* in the infertility centers in Nablus District in the West Bank. *N. gonorrhoeae* infections are expected to be affected by multiple risk factors that vary with lifestyle and culture. Because the latter are difficult to measure, we also designed a questionnaire as a measuring tool to identify these risk factors.

Aims and Objectives

The aim of this study is to find out if there is a significant association between *N. gonorrhoeae* infections and infertility in the Palestinian society, with the purpose of adding screening for these pathogens to the investigation steps of infertility. Specifically, the objectives are:

1. To determine the prevalence of *N. gonorrhoeae* infection among infertile patients in Nablus district in Palestine, and compare it with the fertile group.
2. To determine the risk factors of acquiring infection with *N. gonorrhoeae*.
3. To compare the sensitivities of GC agar selective medium and PCR in detection of *N. gonorrhoeae* in vaginal swab and semen specimens.
4. To make a comparison between fertile and infertile participants with regard to sociodemographic, behavioural, and clinical characteristics.

Materials and Methods

Study Design

The research is a comparative cross-sectional study designed to determine the prevalence of *N. gonorrhoeae* infection among infertile people and compare it to the prevalence of this bacterial species among fertile people in gynecology and infertility clinics in Nablus city, Palestine. The study protocol was approved by the Institutional Review Board (IRB) at An-Najah National University, and verbal consent was taken from each patient after a full explanation of how the sample would be used.

Patients and Specimens' Collection

The study included a group of patients with infertility attending Shunnar Infertility Center and Dima Infertility Center in Nablus city during August 2022 to June 2023. The patients were aged more than18 and smaller than 50 years with no recent use of contraception or antibiotics, and no obvious contributing factor to the infertility including hormonal disorders or genetic syndromes, and for males including varicocele, testicular torsion, hydrocele, or undescended testes. During the same period of time, data and samples were collected from the other group of patients, which included fertile men who visited the infertility centers to provide semen samples for the IVF procedure for sex selection, and fertile women who visited the Gynecology department and clinic at Rafidia Hospital in Nablus city. This group was aged more than 18 and under 50 years, and patients with recent antibiotic use were excluded from the study.

Both fertile and infertile groups were interviewed before collecting their samples. The interview included a full explanation of our research, followed by verbal consent from the patients to collect samples and information from them. It also included a questionnaire about sociodemographic and behavioral features, past medical history, and fertility status.

Vaginal swabs and semen specimens were collected from the included cases. The semen specimens were collected into sterile containers. They were cultured within 1 hour of the sample collection using sterile loops on GC media (Himedia, India), which is selective for *N. gonorrhoeae*. The semen samples were kept in a portable cooler filled with ice until delivery to the lab. There, they were transferred into sterile Eppendorf tubes and stored at -80°C in a deep freezer until DNA extraction.

The Vaginal swabs were taken from the females by using both bivalves speculum and swabs made of a plastic shaft and a rayon tip. The swab used to collect a vaginal specimen was immediately soaked for few seconds with mixing in sterile normal saline placed into a sterile tube with a cap. The collected samples were cultured on GC media and kept at room temperature for 3-4 hours before incubation in an anaerobic jar with 5-10 % CO2. The suspensions made from vaginal swabs were stored in a portable refrigerator full of ice cubes for 3-4 hours until they were transported to the laboratory and placed in sterile Eppendorf tubes and stored at -80℃ in the deep freeze until the time of DNA extraction.

Culture and Gram Stain

The GC agar selective medium (Himedia, India) was prepared according to the manufacturer's instructions. The cultured plates were incubated at 37 ˚C in an environment of 5-10 % CO2 for 72 hours. The plates were examined carefully for growth. The control *N. gonorrhoeae* ATCC 49226 was successfully cultured on the applied GC media.

Each microbial growth on GC agar was examined by the Gram stain according to (13).

Rapid Carbohydrate Utilization Test

This test was carried out according to a previous protocol (13). A bacterial suspension was formed from fresh bacterial culture and then transferred into each sugar-containing well (glucose, fructose, sucrose, and lactose), which were all put in a large well-plate. Each well was mixed properly, and then the well plate was placed in the incubator at 37 ºC for 3 hours, and then a definitive reading was made. Utilization of a sugar was considered positive when a well color turned yellow. However, carbohydrate fermentation test was considered positive for *N. gonorrhoeae* only when glucose-containing wells were positive and the other wells containing other sugars were negative (13).

N. gonorrhoeae ATCC 49226 was also used as a control for this test.

DNA Extraction

DNA extraction from the vaginal swabs was done by taking 1 ml of each sample and centrifuging it at 12000 xg for 10 minutes. The supernatant was discarded, and phosphate-buffered saline with a pH of 7.4 was used to wash the pellet, and then it was centrifuged again. After discarding the supernatant, the pellet was suspended in 50 µL of sterile distilled water. Then the samples were placed in a boiling water bath for 10 minutes and then stored at -20 ˚C before being used for the PCR test (14).

DNA extraction from the semen samples was done using the QIAamp DNA Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions.

The DNA concentration of each sample was measured using a spectrophotometer with a quartz cuvette at 260 nm wavelength.

PCR

The oligonucleotide primer sequences that were applied corresponded to sequences within the porA pseudogene in *N. gonorrhoeae* and were 5′CCGGAACTGGTTTCATCTGATT-3′ and 5′-GTTTCAGCGGCAGCATTCA-3′. The DNA sequences were obtained from Abou Tayoun et al's study (15). In addition, in the optimization of PCR conditions, the previously mentioned reference was used as a guide. Each PCR reaction (50µL) for a single sample consisted of 175 nM of *N. gonorrhoeae* forward and reverse primers, 100 ng/50 µL of DNA template, 3 mM MgCl2, 0.4 mM dNTP, and 2.5 units Taq DNA polymerase. Components of the PCR reaction were obtained from Sigma (USA). DNA extracted from *N. gonorrhoeae* ATCC 49226 was used as a positive control for each run. PCR cycle starts with a step of denaturation for 5 minutes duration at 94°C, then 41 cycles (denaturation at 94°C for 1 minute, 30 seconds of annealing temperature at 60°C, and extension for 1 minute at 72°C). This was all followed by a final step of 5 minutes at 72°C. Afterwards, products were electrophoresed alongside a 100bp DNA ladder (BIO-HELIX, Taiwan) in 1.5% agarose agar and stained with Novel Juice, a DNA staining reagent (BIO-HELIX, Taiwan). A UV transilluminator device was used to observe DNA bands.

Statistical Analysis

In this study, the Statistical Package for Social Sciences (SPSS) for data analysis was used. Demographical characteristics of the samples were presented using tables. The proportion of each study variable and the incidence were calculated. Significant differences between groups were assessed using the Chi-Squared or t-test, as appropriate. Significance level was set at ≤0.05.

Results and Discussion

This study is the first study in the West Bank, Palestine that focused on detection of *N. gonorrhoeae* in semen and vaginal swabs, more specifically among infertile and fertile individuals. This study is important because the majority of *N. gonorrhoeae* infected cases are asymptomatic (8). As shown in Table 1, the subjects were 238 in total, 89 of them were fertile, and 149 were infertile. In addition, 83.1% were females in the fertile group, while 34.2% of the infertile group were males. The mean age of infertile individuals (32.5±7.6) was close to that of fertile (30.7±8.7) ones.

BMI among the fertile group (29.2 ± 4.9) was significantly higher than that of the infertile group (27 ± 5.3). This may be attributed to the increase in body mass of females after pregnancy and delivery of fertile cases (Table 1). This assumption is supported by the finding that the mean of BMI among fertile females (29.2± 4.8) was significantly (P= 0.013) higher than that of infertile females (27.1 ± 5.8), but no significant difference (P=0.109) was found in males. As shown in Table 1, infertility frequency appears to be statistically (0.000) related to the job type. Infertility among unemployed individuals (43%) was significantly (0.000) higher than white collar (22.1%) and blue collar (24.2%), and insignificantly higher than gold collar (6.7%; P=0.103) and others (4%; P=0.699). Unemployment may affect the economic status of individuals, which consequently is reflected negatively on both nutritional (deficiency of vitamins and minerals) and hygiene levels. It was previously reported (16) that fertility falls when unemployment rises.

The level of education was significantly (P=0.013) reflected on infertility (Table 1), where the frequency of infertility among illiterates (8.7%) was significantly lower than that of high school or less (46.3%; P=0.037) and university or more (45%; P=0.047). Similarly, Mary Beth Weinberger (17) reported that there was a decrease in fertility with increasing education.

Among 78 included individuals who live in 48 Lands, 68 (87.2%) suffered from infertility, where this percentage was significantly (0.00) higher than that of individuals living in the West Bank (50.6%). This may be a consequence of the fact that individuals living in 48 Lands were visiting the included IVF centers, which are distant from them, after they were diagnosed with infertility and had tried medication for their fertility problems in 48 Lands. On the other hand, those IVF centers are close to individuals living in the West Bank, and patients visiting them were seeking diagnoses as well as treatment.

Infertile frequency (69.4%) in cases of husbands working in 48 Lands was significantly (0.000) higher than (30.65%) without husbands working in 48 Lands (Table 1). This may be attributed to a long period of absence from mating since workers at 48 Lands may reside away from their families one to several weeks due to the difficulty of transportation between the West Bank and work at 48 Lands. Another possible reason is illegal sexual relationships of workers at 48 Lands and associated infections.

Table (1): Fertile individuals compared to infertile ones with regard to sociodemographic characteristics.

	Fertile Group (n=89)	**Infertile Group (n=149)**
Gender		
Male	15 (16.9%)	51 (34.2%)
Female	74 (83.1%)	98 (65.8%)
Age (mean ± SD)	30.7±8.7	32.5±7.6
BMI (mean ± SD)	29.2 ± 4.9	27 ± 5.3
Education		
Illiterate	2 (2.2%)	13 (8.7%)
High school or less	57 (64%)	69 (46.3%)
University or more	30 (33.7%)	67 (45%)
Job		
Unemployed	68 (76.4%)	64 (43%)
White collar *	5 (5.6%)	33 (22.1%)
Blue collar*	7 (7.9%)	36 (24.2%)
Gold collar*	4 (4.5)	10 (6.7)
Others	5 (5.6%)	6 (4%)
Residency		
The 48 Lands	10 (11.2%)	68 (45.6%)
West Bank	79 (88.8%)	81 (54.4%)
Husband works in 48 Lands		
Yes	18 (25.7%)	68 (69.4%)
NO	52 (74.3)	30 (30.6)
Monthly income (NIS)		
Less than 1350	5 (5.6%)	2 (1.3%)
1350-2490	9 (10.1%)	6 (4.0%)

	Fertile Group (n=89)	Infertile Group (n=149)
2500-3490	13 (14.6%)	23 (15.4%)
3500-5000	29 (32.6%)	55 (36.9%)
More Than 5000	33 (37.1%)	63 (42.3%)

*White collar: office-work jobs (e.g., accountants, computer programmers), Blue collar: construction-works jobs and manual labor (e.g., construction, manufacturing), Gold collar: High-skilled jobs (e.g., Doctors, engineers, lawyers)

Table 2 shows the clinical characteristics of the included individuals. Remarkably, there is a significant association (P=0.000) between infertility and a history of urinary tract infections (UTIs). In more detail, 61.1% had a history of UTIs among the infertile group compared to 36% of the fertile group. This may be explained by the transmission of pathogens responsible for UTIs to the reproductive system, which can cause problems in the reproductive system's function. This result also raises suspicion of occult STIs, which can present with similar symptoms to UTIs (18).

In the Middle East region, in the minds of many, an area where socio-cultural sensitivities surrounding sex and disease still abound (19). The history of STIs was observed to be zero in infertile groups (Table 2). The stigma of STIs among people in Palestine could be a possible cause for not providing true information about a possible history of STIs.

A significant association (0.001) was found between infertility and frequency of condom use (Table 3). It was found that 97.8% of the infertile males had never used condoms compared to 53.3% among fertile ones (P=0.001). This finding supports a possible role of microbial infection in the development of infertility. It's known that condom use reduces the risk of acquiring STIs in males and females (20) and consequently reduces the risk of infertility development. With respect to females, it was reported that persistent use of condoms reduced the risk of recurrent Pelvic Inflammatory Disease (PID), chronic pelvic pain, and infertility (21).

A high percentage (75.2%) of people showed narrow or no knowledge about STIs, even though 40.8% of them had a university or higher education level, and 52.9% had gone to school. Due to the gap between the education level and the percentage of perceived knowledge, we recommend focusing more on STIs education through school curricula or awareness campaigns.

Table (2): Comparison of the clinical characteristics of fertile individuals to those of infertile individuals.

	Fertile (n=89)	Infertile (n=149)	P value
UTI	32 (36%)	91 (61.1%)	0.000
Hypertension	6 (6.7%)	4 (2.7%)	0.182
Hypercholesterolemia	6 (6.7%)	13 (8.7%)	0.585
Endocrine Disease	4 (4.5%)	7 (4.7%)	1.0
Diabetes	4 (4.5%)	1 (0.7%)	0.66
Other Diseases	3 (3.4%)	4 (2.7%)	0.715
Steroid Medication Use	2 (2.2%)	10 (6.7%)	0.219
History of STIs	1 (1.1%)	0 (0%)	0.374
Perceived Knowledge About STIs			
Nothing	44 (49.4%)	83 (56.1%)	0.107
Narrow	26 (29.2%)	26 (17.6%)	
Good	19 (21.3%)	39 (26.4%)	

Table (3): Comparison of behavioral characteristics among fertile and infertile participants

	Fertile (n=89)	Infertile (n=149)	P value
Smoking	22 (24.7%)	37 (24.8%)	0.894
Toilet Type			
Seat	82 (92.1%)	148 (99.3%)	0.003
Non-Seat	7 (7.9%)	1 (0.7%)	
Previous Marriage	8 (9%)	13 (8.7%)	0.945
Current Multiple Partners*	3 (4.5%)	0 (0%)	0.262
Condom Use*			
Never	8(53.3%)	45 (95.7%)	0.001
Sometimes	5 (33.3%)	1 (2.1%)	
Usually	1 (6.7%)	0 (0%)	
Always	1 (6.7%)	1 (2.1%)	
History of being Imprisoned by IOF*	7 (10.4%)	4 (7.8%)	0.755

*for males only.

Also, Table 3 shows that the frequency of seat toilet use by infertile individuals (99.3%) was significantly (0.003) higher than that of fertile individuals. This is mostly due to the increased risk of microbial cross-contamination between seat toilet users.

The results of this study have shown that among the diagnosed types of infertility, 64.5% were primary, 35.5% were secondary, and 42.4% were idiopathic (Table 4).

Table (4): Causes and types of infertility among infertile participants of the study.

	Infertile
Cause of Infertility	
Idiopathic	101(42.4%)
Abnormal Semen Analysis	14 (5.8%)
PCOS	8 (3.4%)
Adhesions	9 (3.8%)
Others	3 (1.3%)
Type of Infertility	
Primary	89 (64.5%)
Secondary	49 (35.5%)

In the present research, GC medium was applied to isolate N. gonorrhoeae from 163 specimens. This selective N. gonorrhoeae medium contains Trimethoprim, Vancomycin, and Nystatin, which inhibit the growth of Gram-negative bacteria except N. gonorrhoeae, Gram-positive bacteria, and fungi, respectively. However, growth of bacteria other than N. gonorrhoeae was detected in 95 out of the 163 (58.3%) tested specimens (Table 5). This indicates that the bacteria are becoming more resistant to the potent broad-spectrum antibiotics. In parallel to our results, increased resistance rates to commonly used antibiotics have been reported by Adwan et al (22).

The bacteria detected on GC medium were examined by Gram stain, where Gram-negative cocci were detected in 16 cases. All Gram-negative cocci were examined by the rapid carbohydrate utilization test and the oxidase test. All were oxidase positive; however, only 2 isolates possessed carbohydrate results characteristic of *N. gonorrhoeae*. They were not confirmed by a PCR test of the culture. Therefore, the culture approach did not detect any *N. gonorrhoeae* bacterial isolates. PCR was used to examine all of the collected 238 specimens to detect *N. gonorrhoeae*, where PCR was positive in 2 cases that were negative by culture. Therefore, PCR appears more sensitive than culture for detecting *N. gonorrhoeae*. In another study, it was mentioned that PCR is more sensitive than traditional methods, such as culture, for detecting *N. gonorrhoeae* (23).

Based on PCR results, the prevalence of *N. gonorrhoeae* infection among the 238 studied cases was 0.8%. Furthermore, *N. gonorrhoeae* was detected in 1 case of fertile females (1.1%) and 1 case (0.7%) of the infertile group (P=1.00) (Table 5). Similarly, in a study carried out in Australia (24), genital gonorrhoea was detected in 142 (1.1%) cases of the visits at a sexual health service in Melbourne. Almost half of the cases were asymptomatic. However, a higher prevalence (3.28%) of *N. gonorrhoeae* infection among reproductive-aged women in sub-Saharan Africa was found using meta-analysis of 35 studies (7). In a meta-analysis and metaregression study, 147 studies of gonorrhoea prevalence among infertile case studies were identified from 56 countries. The pooled mean prevalence of current gonorrhoea infection among infertile patients was estimated globally at 2.2%, with the highest prevalence in Africa

at 5.0%. The mean prevalence was higher for populations with tubal factor infertility (3.6%) and mixed cause and unexplained infertility (3.6%) compared with other diagnoses, such as ovarian and non-tubal infertility (0.1%), and for secondary (2.5%) compared with primary (0.5%) infertility (25). In our study, only one *N. gonorrhoeae* was detected in a male patient suffering from primary infertility.

Isolated bacteria from fertile and infertile individuals were compared (Table 5). Microbial growth on GC medium was observed in 69.7% of specimens collected from the fertile group compared to 45.1% in the infertile group.

Table (5): Results of culture, Gram stain, and PCR of the examined specimens.

Culture results for 163 specimens		
	Fertile (n=89)	**Infertile (n=74)**
Microbial growth on media	62 (69.7%)	33 (44.6%)
Gram stain and morphology		
Gram- positive cocci	28(31.5%)	9(12.2)
Gram-negative cocci	2(2.2%)	8(10.8%)
Gram- positive Bacilli	12(13.5%)	3(4.1%)
Gram-negative Bacilli	5(5.6%)	6(8.1%)
Mixed	13(14.6%)	6(8.1%)
Fungus	2(2.2)	1(1.4)
PCR results for 238 specimens		
	Fertile (n=89)	Infertile (n=149)
N. gonorrhoeae	1 (1.1%)	1 (0.7%)

Conclusion

Examining urogenital specimens by PCR for *N. gonorrhoeae* showed a more sensitive approach than the culture method. The presence of *N. gonorrhoeae* infection is not significantly associated with infertility. Infertility is significantly associated with the history of UTIs, never using condoms, the frequency of use of a seat toilet, the husband working in 48 Lands, and unemployment. Bacteria other than *N. gonorrhoeae* are becoming more resistant to the potent broad-spectrum antibiotics. It's strongly recommended that the study be repeated, including a larger number of participants from infertility centers in different districts in Palestine, as well as to determine if there are molecular and antibiotic resistance differences between strains isolated from fertile and infertile individuals.

Limitations

The collection of the samples was very difficult and limited for various reasons. To mention some, access to the infertility centers was hard, and we were rejected by plenty of centers in the country. Even when we were accepted, it was hard to continue the collection process because of the center's protocols or people's refusal to participate. All of this is due to the lack of perception of the importance of medical research and the sensitivity of this research topic due to cultural values. There were only 2 cases of the disease discovered out of all the samples because the society in the Nablus district is a conservative one, since transmission of gonorrhoea disease is mainly facilitated by having many sexual partners. The limited number of *N. gonorrhoeae* positive results hindered the detection of possible *N. gonorrhoeae* infection related to infertility or other variables.

Disclosure Statement

- **Ethical approval and consent to participate:** Ethical approval was obtained from the Institutional Review Board (IRB) at An-Najah National University. Informed consent was attached to every clinical visit, and the participants were informed that their participation in the study was voluntary. They were also informed about the study process and sample collection, including benefits and side effects. Any development of adverse effects was dealt with immediately and efficiently as recommended, and participants could withdraw at any time without declaring the reason. Participants' privacy and confidentiality were assured.
- **Consent for Publication:** Not applicable
- **Availability of data:** Data used for this study will be made available upon request**.**
- **Author's contribution:** Dr. Motasem Y. Al-Masri and Dr. Mohammad Qadi were involved in design, certain research methods, and writing. Aya Mikkawi, Safaa Abatli, Mohammad Abdulhalim, and Dana Ajoli were involved in specimen collection, research methods, and writing. Dr. Zaher A. Nazzal was involved in designing the study, statistical analysis of the data and writing. Abd Razak Zarour was involved in certain research methods.
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