Profiling the Bacterial Infection-Infertility Nexus Among Clinical Populations of Tripoli-Libya

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Abstract

Infertility is the inability to conceive after one year of regular unprotected intercourse, or six months for women aged 35 years and older. It can be defined as the inability to achieve pregnancy without medical intervention. Affecting 15% of couples globally, bacterial infections can contribute to infertility, affecting one in seven couples in the West and one in four in developing nations. Infertility rates can reach 30% in regions such as South Asia, sub-Saharan Africa, the Middle East, North Africa, Eastern Europe, and Central Asia. Various factors, such as congenital and hormonal disorders, lifestyle changes, stress, and environmental factors, can contribute to infertility. This study aimed to examine the link between infertility and bacterial infection in Tripoli, Libya, by analysing medical records from the National Infertility Centre and private labs. It seeks to identify the most common bacteria associated with fertility issues and assess the impact of bacterial infections on infertility, considering factors such as PCOS. The prevalence of infertility in both men and women was also determined. A total of 302 medical files of 151 males and 151 females from 2023-2024 were analyzed. Risk factors such as hypertension, diabetes mellitus, and smoking were also explored across different age groups. Statistical analysis using SPSS software revealed the relationship between infertility and bacterial infections, which are presented in tables, frequencies, and P-values. However, this study revealed that 80.1% of the studied patients were infertile. Of the infertile cases, 39.4% had bacterial infections, 19.9% were not infertile, and 123 (40.7%) had non-bacterial infections or infertility. Based on the collected data, this study concluded a strong correlation between infertility and bacterial infection in males (81.5%) compared to females (18.4%), with a P-value (< 0.05).

Keywords: Infertility, Bacterial Vaginosis, Lactobacilli, H. Pylori, Microbiota.

Introduction

Infertility is defined as the inability to achieve pregnancy and/or the need for medical intervention to achieve a successful pregnancy[1]. Infertility is a complicated illness that affects 15% of couples across the globe, and bacterial infections are known to play a major role in its development, affecting approximately one in seven couples in the Western world and one in four couples in developing countries. Fertility rates may reach 30% in certain regions worldwide, such as South Asia, some countries in sub-Saharan Africa, the Middle East, North Africa, Central and Eastern Europe, and Central Asia; males are solely at fault for 20-30% of infertility cases, while globally they contribute to half of all cases [2].

Infertility can affect both sexes due to various conditions, such as hypogonadotropic hypogonadism, hyperprolactinemia, cystic fibrosis, infections, and lifestyle factors [3]. Although bacterial infections can affect male fertility by affecting various parts of the male reproductive system, such as spermatogenesis, some bacteria, such as Escherichia coli, Chlamydia trachomatis, and Ureaplasma urealyticum, can reduce sperm production by causing inflammation and oxidative stress in the testes and epididymis. Bacterial infections can lead to reduced sperm movement, abnormal sperm shape, and damaged sperm DNA, which may hinder fertilization chances. Bacteria have the potential to lead to infections in the male reproductive system, which can manifest as epididymitis, prostatitis, and orchitis, leading to inflammation, scarring, and potential blockage of the reproductive tract. E. coli, S. aureus, C. trachomatis, U. urealyticum, N. gonorrhoeae, and P. aeruginosa are frequent bacteria linked to male infertility [4].

Studies have found that the microbiota of the female reproductive tract in endometriosis is distributed similarly in the cervical mucus of women with and without endometriosis, regardless of the phase of the menstrual cycle; however, lactobacilli are the most common species found in the vagina and cervix. Moreover, the presence of Corynebacterium, Enterobacteriaceae, Flavobacterium, Pseudomonas, and Streptococcus was elevated in the endometriosis group compared to that in the control group, with Enterobacteriaceae and Streptococcus being the most prominent examples[5]. Nevertheless, BV is characterized by an unequal balance in the vaginal microbiota, marked by a reduction in lactobacilli and a rise in anaerobic bacteria such as Gardnerella vaginalis, which is strongly linked to infertility of the fallopian tubes and can negatively affect the success rate of assisted reproductive technologies (ARTs). Studies have

shown that women with BV have a higher chance of experiencing unfavourable reproductive outcomes post in vitro fertilization (IVF)[6, 7]. A decrease in lactobacilli dominance can lead to a higher risk of sexually transmitted infections (STIs) and upper genital tract infections as bacterial pathogens and anaerobic bacteria ascend, furthermore, BV is linked to changes in the ideal vaginal microbiota, indicated by lower levels of bacteria that produce lactic acid and higher levels of various anaerobic bacteria, Gardnerella vaginalis, Megasphaera spp., Atopobium vaginae, Dialister spp., Mobiluncus spp., Sneathia amnii, Sneathia sanguinegens, Porphyromonas spp., and Prevotella spp. are frequently linked to BV, while BV often has no symptoms [8].

BV is a leading cause of vaginal dysbiosis in women of childbearing age, leading to abnormal vaginal discharge with a fishy smell; however, symptoms can vary or be absent. It is estimated that the prevalence of BV varies from 5% to 15%, potentially reaching 30-40% among women of reproductive age. According to research conducted by Salah et al. (2013), there was a notable difference in the prevalence of infertility and BV between fertile women (15.4%) and those who were not (45.5%); furthermore, BV was found in 60% of PCOS patients and over 35% of women with unexplained infertility have BV [9]. Hong et al. (2021) found that women with PCOS, regardless of testosterone levels, had higher levels of Gardnerella, a bacterium associated with Bacterial Vaginosis (BV). This finding suggests a higher likelihood of BV in women with PCOS. Hong et al. also noted a connection between testosterone levels and certain bacterial populations in women with PCOS. Lu et al. (2021) found a link between FSH levels and the number of Lactobacillus spp. present. A decrease in Lactobacillus spp. in the vaginal microbiome of women with PCOS can lead to an increase in harmful microorganisms such as Gardnerella vaginalis, Prevotella, Mycoplasma, and Chlamydia trachomatis. Gu et al. (2022) studied the impact of menstrual cycle length on the reduction of Lactobacillus spp. and subsequent growth of pathogenic microorganisms in PCOS patients, ultimately, PCOS may elevate the risk of BV and reproductive issues by disrupting the balance of bacteria in the vaginal microbiome [9]. A decrease in bacterial diversity and richness is a characteristic feature of dysbiosis in the microbiota of the male reproductive tract, a decrease like this can upset the equilibrium of microbial communities, leading to male infertility issues, increase in harmful organisms; Imbalance in bacterial composition can result in a build-up of pathogenic species in the male reproductive system, bacteria such as Escherichia coli and Ureaplasma urealyticum have been linked to chronic prostatitis and inflammation, these species can cause oxidative stress, inflammation, and harm to the male reproductive system, which can affect sperm quality and fertility [10]. A study by Ramlau-Hansen et al. from 1987 to 2004 found that smokers had lower semen volumes, sperm counts, and motile sperm than non-smokers, and the link between smoking and sperm concentration was dependent on the number of cigarettes smoked, with those smoking more than 20 days experiencing a 19% decrease, while another study on 1786 men for infertility further supported these findings, showing that smoking in adults led to a moderate decline in semen quality [11]. Zhang and his colleagues studied 362 Chinese male patients at a fertility centre and found that smokers had lower semen volume, sperm concentration, and motility compared to non-smokers. They also investigated the role of

superoxide dismutase, an enzyme related to oxidative stress, and found lower levels in infertile men who smoke, another study of 200 infertile men showed similar results with decreased sperm movement and abnormal sperm shape in smokers, as well as, Chia et al confirmed these findings in a group of 618 Chinese males, showing a correlation between smoking and lower sperm concentrations and higher rates of abnormal sperm morphology based on the amount smoked [12].

E. coli (15% infertility cause worldwide) is the most common bacterium found in the semen of men with infertility issues, where, outer membrane vesicles (OMVs) are continually produced by all Gram-negative bacteria in which, the study related to E. coli and infertility for the first time, demonstrated the role of OMVs in human sperm function. OMVs play a role in altering human sperm function through two processes:(1) reduced motility and (2) DNA fragmentation [13]. In 1994, Monga et al. discovered that there is a receptor-ligand interaction between fimbriated E. coli and spermatozoa, which causes sperm agglutination and reduces motile sperm count, which could be glycoproteins such as type 1 fimbriae or glycolipids such as P-fimbriae. Additionally, reports indicate that the surface of sperm cells contains a high amount of glycoproteins; therefore, the asymptomatic presence of Enterobacteriaceae in the male or female genitalia could lead to comparable interactions [3].

Kaur and Prabha et al. were the first to discover the sperm agglutinating factor (SAF) After noticing that S. aureus can stick to both the head and tail of sperm, and clump together mouse sperm cells, the team successfully separated a 57 kDa protein from S. aureus and connected this protein to the regulation of sperm movement and viability, additionally, SAF has the potential to impact different sperm parameters including Mg2+-dependent ATPase activity, acrosome status, and apoptosis, to back this up, a significant change in shape happens in the sperm cells when they attach to SAF, as observed through scanning electron microscopy [14]. Momoh et al. found that Staphylococcus aureus was the most prevalent microorganism in the semen culture of infertile men, with a prevalence rate of 75%[15]. Recently, Ruggeri et al. found that S. aureus is a common contaminant in both male and female genital fluids, another research study indicated a notable reduction in sperm movement and clumping when exposed to S. aureus, along with testing a 70

KDa purified factor in animals intravaginal for 10 days leading to infertility [1, 3, 16]. S. haemolyticus leads to infections in the male reproductive system and may result in male infertility in addition, infection from S. haemolyticus leads to a reduction in both sperm motility and viability, moreover, interactions between S. haemolyticus and ejaculated sperm can influence the structure of the sperm cell membrane, potentially resulting in male infertility [16].

H. pylori, a microaerophilic, gram-negative, spiral-shaped bacterial species, is the type species of the Helicobacter genus, specializing in infecting humans in the gastroduodenal tract, it plays a crucial role in causing peptic ulcers and are key elements in the formation of gastric tumours, the Japanese group first suggested the link between H. pylori infection and infertility in women in 2002, finding a higher prevalence in female patients with fertility issues compared to controls (P = 0.033), Moreover The existence of certain antibodies in various parts of the female reproductive system may play a role in causing problems, as they could impact sperm movement and the process of sperm becoming capable of fertilization, which is necessary for successful fertilization, however, H. Pylori with male infertility associated with Sperm cells being the sole flagellated cells in humans; the flagella of sperm may have a linear similarity with bacterial flagella since structures with similar functions are typically preserved throughout evolution. In men experiencing fertility issues, concomitantly, increased systemic levels of tumor necrosis factor-alpha (TNF-a), a pro-inflammatory cytokine that may cause sperm damage, were observed in a group of idiopathic infertile males infected with H. pylori strains expressing CagA [17].

Infertility affects millions of people across the globe, which impacts families and communities from a social perspective, and is responsible for 15% of couples' illnesses worldwide. The aetiology of infertility related to infertility is numerous, including psychological, physiological, and microbiological disorders; however, there is little concern regarding microbiological factors. Therefore, this study aimed to investigate the relationship or the association between infertility and bacterial infection in Tripoli, identifying the most predominant bacteria that relate to fertility disorders by reviewing medical file datasets collected from the National Infertility Center (NIC) and other private laboratories in the Tripoli district.

Methods Study area

The study was conducted at the National Infertility Centre in Tripoli and other private laboratories (Alandalus Laboratory and Al-Amal Laboratory).

Study design and population

A retrospective study design was used, which involved studying 302 medical files of 151 males and 151 females between 2023 and 2024 to investigate the relationship between infertility and bacterial infection within various age groups, as well as risk factors such as hypertension, diabetes mellitus, and smoking.

Data collection

The data of archived medical files were collected on three separate days, which involved 302 cases of female and male patients combined within variables addressed as sex, age, medical diagnosis, infertility, and bacterial type with resident of patient.

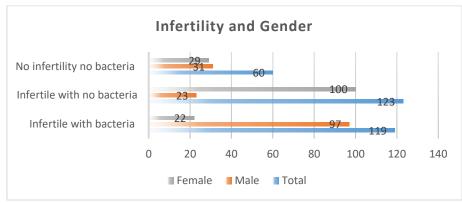
Statistical analysis

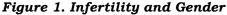
Data were collected and statistically analysed using SPSS software (V20.0SPSS). The result of data was given in the form of rates expressed as a percentage; it was presented in tables. The chi-square test was used to measure the association between the variables where the P-value was less than 0.01, which was considered highly significant.

Results

In this study, which comprised 151 males and 151 females, 302 females and males combined in total, from 302 cases, 242 individuals' cases identified with infertility represented 80.1%, while 60 individual cases were identified with no infertility, representing 19.9% (Figure 1). In addition, from 242 cases, 119 individuals identified with Yes (including bacterial infection) represented 39.4%, while 123 individuals identified with Yes (no bacterial infection) represented 40.7%, which may suggest other causes such as, PCOS (polycystic ovarian syndrome, hypertension, smoking, Diabetes Mellitus and Thyroid dysfunction (Hypothyroidism or Hyperthyroidism).

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In terms of bacterial type, E. coli was the predominant bacteria in the dataset with a score of 47, followed by S. aureus with a frequency of 41 among both sexes; however, the main focus was precisely the male part, since males show a higher percentage of bacterial infection in comparison to females, which is approximately 14.5%, while males were 64.2%. Nevertheless, the most predominant bacterial infection in females was Lactobacillus BV (6.6%), followed by S. aureus and E. coli (5.2% and 0.6%, respectively). S. haemolyticus was also noticed in the statistical analysis, which represents 5.6% for both genders. Interestingly, the presence of H. pylori in the female section, although its percentage is low, may suggest that H. pylori may contribute to fertility disorders (Figure 2).

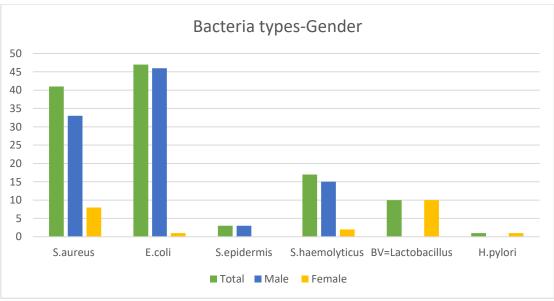


Figure 2. Bacteria types.

From the chart bar above, if the data are extracted and entered into demographic data, we will find that there is a significant variation in the number of bacterial infections related to infertility. Regarding bacterial type, It was found that S. aureus and E. coli were the major dominant bacteria among males with 33 and 46 males respectively that represents 80.5% and 97.9%, whereas, S. aureus and E. coli were 8 and 1 respectively among females that represents 19.5% and 2.1%. Subsequently, BV (Lactobacillus) was the major bacterial species found among females, with 10 following S. haemolyticus and H. pylori with 2 and 1, respectively, representing 100% for BV, 13.3% for S. haemolyticus, and 100% for H. pylori.

The percentage of S. haemolyticus was higher in males (15 out of 17), representing 88.2%. In relation to infertility with no bacteria, it was found on the dataset that a total of 125 were infertile, with no bacteria constituting 100 females representing 80%, while males showed 25 out of 125, representing 20%. This indicates that the majority of infertility cases among females were not due to bacterial infection, which may suggest other causes such as polycystic ovarian syndrome (PCOS), Hypertension, Thyroid dysfunctions, which consist of hypo-and hyperthyroidism, and diabetes mellitus. In addition, this result shows a higher percentage of bacterial infection in males than females, and this can be displayed from the demographic data in which a total of 119 of both infertile genders with bacteria consisted of 97 males (81.5 %) and 22 females (18.5 %). All statistical information can be found in the demographic tables. Regarding infertility,

there was a significant correlation between infertility and bacterial infection, which can be observed from the P-value that was less than 0.01 (< 0.01).

From the demographic data, in terms of age group, it was found that the estimated mean age 42.5 with (+) or (-) 54.4 SD, which indicates that the mean lies within the category of 41-60 years (95%CI 38.263, 46.733; < 0.05) between 20 and 60 years, which consisted of 151 males and 151 females categorized into three different categories, as mentioned in the demographic table, which portrays a total of 150 of the 20-40 group that has 55 males and 95 females between 20-40 years, whereas, a total of 114 of 41-60 has 65 males and 49 females belongs to the 41-60 category. Finally, the last category is 61-80 with a total of 38, consisting of 31 males and 7 females. There was a significant correlation between age and sex as well as age and infertility, which can be shown with a p-value < 0.05, indicating high significance or correlation that shows a strong relationship between age and gender as well as age and infertility (Figure 3).

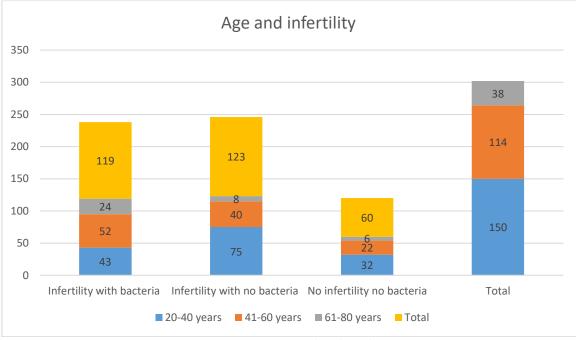


Figure 3. Age and infertility

The data in this chart illustrate the number of individuals with infertility. Hence, it illustrates the category of 20-40 that has a total of 119, which consists of 43(36.1%) individuals being infertile with bacteria as well as 52(43.697%) individuals in the 41-60 category, while 24 (20.168\%) individuals belong to the 61-80 category. Those with (yes with no bacteria) category showed 123 being 75 (60.9%) individuals with 20-40, whereas 40(32.52%) individuals in the 41-60 category while 8 individuals (6.58%) with 61-80. Finally, the category of (No), which shows 32 individuals (53.4%) with 20-40, while 22 individuals (36.6%) belong to the 41-60 category, whereas, 6 individuals (10%) belonged to the 61-80 category.

Of the total 150 individuals in the 20-40 category, 43 (28.7 %) had bacterial infertility, 32(21.3%) had no bacteria, and 75(50%) had infertility but no bacterial presence. A total of 114 individuals in the 41-60 category displayed 52(45.6%) individuals with bacterial infection and 40 (35.0\%) individuals with infertility but no bacterial infection, while 22(19.3%) individuals had no bacterial infection and no infertility. A total of 38 of the 61-80 categories show 24(63.2%) individuals with infertility related to bacterial infection, while 8(21.05%) individuals showed infertility with no bacterial infection; however, 6 (15.79\%) individuals showed no infertility or bacterial infection.

Clinical diagnosis from the demographic data showed a p-value of less than 0.05, which suggests that clinical signs may contribute to infertility disorders. Clinical signs shall be illustrated in this chart

In the chart above, it shows various clinical signs that influence fertility function negatively, one of these signs which is the highest bar that indicate (Menarche plus HTN and DM) which represents 14.2% then menarche alone with whereas, HTN, DM, both separately as well as, HTN, DM and PCOS shows 9.9%,12.9% and 8.6% respectively however, on the male part, HTN and being heavy smoker as well as, Varicocele with 9.3% and 8.6% shows high percentages in terms of male clinical conditions, these signs can be found frequently when diagnosis of infertility of male and female is taken place.

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Table 1. Demographic data							
Parameters	Mean age ± SD	Total N=302 (100%) 42.5(±)54.4	Male N=151 (50%), 46.6, (±)16.77	Female N=151 (50%), N=152 48.8, 24.72(±)	Chi-square test (t/x2)	P-value	95% CI
Patient Group	Infertile with bacteria	119	97	22	95.539	<0.05	0.838, 1.74
	Infertile with no bacteria	123	23	100			
	No infertility no bacteria	60	31	29			
Age group	20-40	150	55	45	25.190	<0.05	38.3, 46.3
	41-60	114	65	49			
	61-80	38	31	7			
Bacteria type	S. aureus	41	33	8	127.270	<0.05	0.47,0.67
	E.coli	47	46	1			
	S. epidermis	3	3	0			
	BV=Lactobacillus	17	15	2			
	H. pylori	1	0	1			
Clinical conditions	Hypertension	42	21	21			
	DM	42	21	21			-
	HTN, DM and PCOS	26	0	26			
	Oligozoosermia	1	1	0		270.44 < 0.05 1.20,1.81	
	Menarche plus HTN and DM	43	0	43			
	Endometriosis	1	0	1			1.20,1.81
	Azoospermia	1	1	0			
	Varicocele	26	26	0			
	Hyperthyroidism	3	2	1			
	HTN plus heavy smoker	28	28	0			
City of Origin	Tripoli	101	60	41	22.985	> 0.05	0.332 ,0.751
	Janzour	81	34	47			
	Abu Salim	10	6	4			
	Tajoura	47	25	22			
	Ain-Zara	28	19	9			
	Qasir Bin Ghasir	21	5	16			
	Zawia	10	9	1			
	Zawara	4	3	1			

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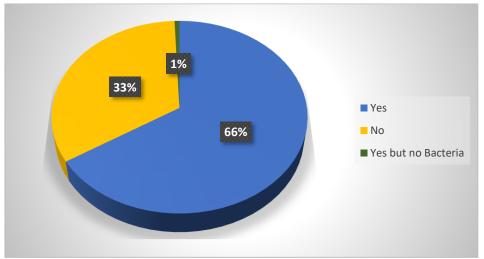


Figure 4. Infertility categories

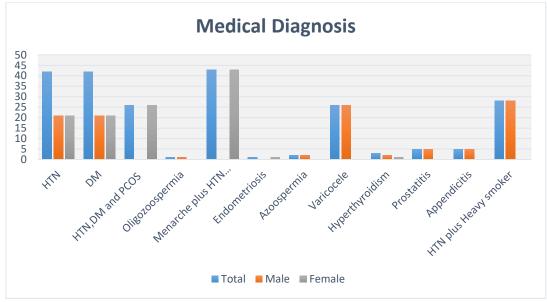


Figure 5. Medical diagnosis among both genders.

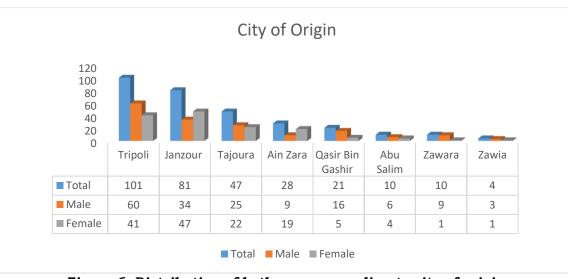


Figure 6. Distribution of both sexes according to city of origin.

The city of origin was also included in this study, which shows individuals from various locations who are infertile or not infertile. This is illustrated in the following chart. In the chart below, it shows the highest city of infertility being found greatly in which Tripoli was placed the first on the rank that displays 33.4%

following by Janzour with 26.8%, Tajoura with 15.6%, Ain Zara with 9.3%, Qasir Bin Ghasir with 7%, Abu Salim with 3.3%, Zawara with 3.3% and Zawia with 1.3%. However, when it comes to distinguishing these data to explore the number of males and females from this data, we found that a total of 101 individuals, comprising 60(59.4%) males and 41(40.59%) females belonging to Tripoli, with a total of 81 male and female individuals constituting 34(41.98%) males and 47(58.02%) females belonging to Janzour. Tajoura was found with a total of 47 individuals, which consist of 25(53.19%) males and 22(46.80%) females, while Ain-Zara was found with a total of 28 individual, whichat is comprised of 9 males and 19 females. Qasir Bin Ghasir was found with a total of 21 individu: 5 males (23.8%) and 16 females (76.19%). Abu Salim was a total of 10 individuals with 6 (60%) males and 4 (40%) females. A total of 10 individuals, 9(90%) males and 1(10%) female, belonged to Zawara. Finally, Zawia had a total of four individuals: three (75%) males and one (25%) female.

Discussion

In this study, which was conducted to find the association or the correlation between infertility and bacterial infection among Libyans, some studies agree on the founding we have, while others contradict the findings we have; for example, the most underlying factor that causes infertility was the menstrual cycle in which menarche was the most common finding in this study, which agrees with the study being published in India [18].

In a comparison to a study published in India, it was found that the female factor was the dominant factor over the male factor, in which the female factor represents 46.68%, whereas the male factor represents 9.16%, although this statement agrees with our study findings in which male factors, regardless of whether there is a bacterial infection, were represented in higher percentages, which were 79.47% for males and 80.79% for females [19].

In males, it was found that azoospermia and oligozoospermia were attributed to infertility disorders in males, in which a study from Turkey agreed with the statement mentioned, although the percentages were not high enough; in turkey, it was reported that the percentage was 32% of azoospermia and oligozoospermia, whereas in our study, it was 1.9% related to azoospermia and oligozoospermia combined. Several studies have reported that the global prevalence of PCOS ranges from 3 to 10%. However, the prevalence varies depending on the criteria used, with the NIH estimating around 6% and the Rotterdam criteria and Androgen Excess Society guidelines at 10%. A recent study in a random sample of people from Qatar found that 12.1% of the population had PCOS according to NIH guidelines, potentially rising to 20% under Rotterdam or Androgen Excess Society criteria, a notably higher rate than in women from different continents and ethnic backgrounds. In comparison to our findings, PCOS along with HTN and DM constituted 17.2% of the female population in our study, which represents the highest percentage of medical diagnoses among Libyan females. Studies in Nigeria and Uganda reported that varicocele was the leading cause of male infertility (49.4%), with azoospermia (56.4%) and oligozoospermia (24.5%) being the most common factors identified during semen analysis. However, in our study, varicocele contributed 17.2%, which came second after hypertension plus heavy smokers (18.5%), although the percentage was lower than that in the Uganda and Nigeria studies [20].

Age and infertility in the this study, shows various percentages which can be shown as 78.6% for 20-40 category, 80.7% for 41-60 and 84.2% for 61-80 category In comparison to study conducted in Barcalona-Spain, illustrating that fertility declines when the age increases between 30-35 years, although the study that I conducted was organized in different category, however it shows similar results regardless of different categories[21]. Interestingly, H. pylori is found in the female population, although it was not very common in this study. This presence may indicate a possible link between H. pylori and fertility issues, which can be seen in a study published in Japan that was mentioned earlier, which stated that there is a significant correlation between H. pylori and H. pylori since the P-value is less than 0.05 [17].

Conclusion

There was a strong association between infertility and bacterial infection, precisely among male individuals due to the high percentage, which was 81.5%, whereas females exhibited 18.4% of infertile bacterial infections.

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المستخلص