The Egyptian Journal of Hospital Medicine (July 2021) Vol. 84, Page 2279-2284

Effect of Genital Tract Infection on Citric Acid in Semen of Infertile Male Patients

Doaa Abdel Rahman Sonbol, Ahmed Fathy State, Samir Mohammad Elhanbly
Department of Dermatology, Andrology and STDs - Faculty of Medicine -Mansoura University, Egypt
***Corresponding author:** Ahmed Fathy State, **Mobile:** (+20) 01001409323, **E-Mail:** ahmedelkordys4@gmail.com

ABSTRACT

Background: Infertility means the failure to achieve a clinical pregnancy after 12 months of regular unprotected sexual intercourse. It affects approximately 15% of couples. The male factor is the main cause of infertility in 20% of cases and contributes in about 50%. There are many etiologies for male factor infertility. Infectious processes contribute to about 15% of such cases. Citric acid has antioxidant and anti-inflammatory functions in tissues damaged by environmental factors. In addition, it favors the synthesis of glycosaminoglycans in various tissues. Citric acid levels are regulated by testosterone, and like fructose can be elevated in oligozoospermic and azoospermic subjects without a convincing clinical explanation.
Objective: To evaluate effect of genital tract infection on level of citric acid in semen of infertile men.
Patients and methods: This study was carried out on 30 infertile male patients. Patients were recruited from the Outpatient Clinic of Andrology Unit in Dermatology & Andrology and STDs Departments, Mansoura University Hospital for one year.
Results: The mean age of the studied group was 29.57 ± 4.22 years. All studied samples were subjected to antimicrobial sensitivity tests. The most sensitive antibiotics were rifampicin, cefoprazone/sulbactam, amoxicillin/clavulanic acid and piperacillin/tazobactam. While the most resistant antibiotics were ciprofloxacin, cephalexin and levofloxacin. The mean of citric acid was significantly increased after receiving antibiotic (16.64 ± 2.24 versus 3.32 ± 1.40 mg/ejaculate before treatment).
Conclusion: Citric acid was significantly increased after receiving antibiotic versus before male genital infection treatment.
Keywords: Sexually transmitted diseases, Genital tract infection, Infertility, spermatogenesis.

INTRODUCTION

Infertility means the failure to achieve a clinical pregnancy after 12 months of regular unprotected sexual intercourse. It affects approximately 15% of couples [(1)]. The male factor is the main cause of infertility in 20% of cases and contributes in about 50% [(2)]. There are many etiologies for male factor infertility. Infectious processes contribute to about 15% of such cases [(3)]. Male genital tract infections are difficult to detect as they are asymptomatic in many cases [(4)]. A number of patients seeking treatment for impaired fertility are increasing so the diagnosis of "silent" genital tract infections should receive attention as the infection may be linked to asthenozoospermia [(5)]. Infections are potentially treatable causes of male infertility, but the resistance to common antibiotics and the poor compliance may impede the efficacy of antibiotics in resolving complicated GTI or restoring fertility [(6)].

In a study on 140 patients with pyospermia, 92 of them (65.7%) yielded bacterial growth with Staphylococcus aureus, Streptococcus saprophyticus and Escherichia coli with the highest incidence rate by 28.3%, 19.6% and 13.0% respectively. Then, there were Proteus mirablis, Klebsiella pneumonia and Proteus vulgaris with 10.8% for each. Pseudomonas aeruginosa was 5% [(7)].

Bacteriospermia affects the normal fertility process by any of the following mechanisms: deterioration of spermatogenesis, decreased sperm motility, altered acrosome reaction, altered morphology, formation of reactive oxygen species leading to increased DNA fragmentation index, formation of antisperm antibodies due to breach in the blood-testes barrier, and genital tract obstruction due to inflammation and fibrosis [(8)]. The Polymorphnuclear leukocytes (PMN) count is considered the best known marker of inflammation in the genitourinary tract and are a routine diagnostic parameter [(9)]. Other inflammation marker includes citric acid (a prostate gland function parameter) [(10)]. Citric acid has antioxidant and anti-inflammatory functions in tissues damaged by environmental factors. In addition, it favors the synthesis of glycosaminoglycans in various tissues. Citric acid levels are regulated by testosterone, and like fructose can be elevated in oligozoospermic and azoospermic subjects without a convincing clinical explanation [(11)].

The importance of citric acid in altering sperm attributes during abstinence has been underrated. Inspecting the levels of citric acid in seminal plasma thus may benefit to find the probable causes of male infertility. A proper counseling (brief medical history, physical inspection and imaging), simple semen analysis beside biochemical evaluation of seminal plasma is the prerequisite step to identify potential cause affecting viability, motility and morphology of spermatozoa [(12)].

The aim of this study was to evaluate effect of genital tract infection on the level of citric acid in semen of infertile men.

Received:23 /4 /2021
Accepted:19 /6 /2021

PATIENTS AND METHODS

This study was carried out on 30 infertile male patients. Patients were recruited from the Outpatient Clinic of Andrology Unit in Dermatology & Andrology and STDs Departments, Mansoura University Hospital for one year.

Inclusion criteria: Infertile men with pyospermia and age group from 20 to 60 years old.

Exclusion criteria: Unmarried, moderate to severe varicocele, cigarette smoking and drug abuser, and patients who received antimicrobial 3 months ago.

All participants in this study were subjected to the following:

1. Full history taking (sexual, martial history, previous medications and interventions).
2. Complete general and andrological examinations.
3. Collection of semen samples from infertile male patients by masturbation after 2 to 5 days of abstinence. Before sampling, the patients were advised to urinate and then wash their glans penis with regular soap and water then dry it with clean towel. Also, the patients were carefully instructed to avoid contamination of inner jar by fingers or the penis.
4. Semen analysis was done with computer assisted semen analysis (CASA).
5. After detection of pyospermia by peroxidase stain, citric acid was measured in semen using citric acid test.
6. The citric acid test works as follow in two steps: Spermatozoa and particles are removed by isopropanol (causes precipitation) and after centrifugation; ferric chloride is added to the supernatant. The Fe^{3+} ions and citrate form a complex that turns the solution to a yellow color. The intensity of the color is directly related to the amount of citrate and can be measured in a photometer or plate reader. The Materials included in the kits are Reagent 1 - 28ml FeC13 solution (Foams easily: Do not shake), Reagent 2 - 1 1 ml Isopropanol, and Reagent 3 - 2ml citric acid standard (4mg/ml). The test is preferably done on (frozen/thawed) semen plasma instead of the whole semen sample to avoid the formation of excessive precipitation during the analysis. Freeze seminal plasma if the sample cannot be tested at the same day of ejaculation.

   Methods:

   Assessment of semen sample preferably to be done at the same day of ejaculation or a subsequent day if samples were frozen. Semen sample was prepared by adding 100 ul of reagent 2 to 100 ul of semen or semen plasma and mix gently. Standard: add 30 ml of reagent 2 to 30 ml of standard solution and mix gently. Blanc sample: add 30 ml of reagent 2 to 30 ml of purified water and mix gently. Centrifuge for 20 minutes at 2500 rpm (This resulted in precipitation of spermatozoa and other particles present in the semen sample). Pipette 25 ml of standard and Blanc sample in an empty well. For the semen sample, carefully pipette 25 ul of supernatant into an empty well as follows: Place pipet tip in the middle of the Eppendorf tube, right below the meniscus of the supernatant, and slowly move pipet tip downwards whilst aspirating fluid. Remove supernatant very carefully in order not to aspire any of the sediment. In case the supernatant is turbid, centrifuge again at 2500 rpm for another 10 minutes. Add 200 pl of reagent 1 to the well slowly. Mix gently to avoid air bubbles. Read the results of the samples and the standard at 405 nm. To obtain total citric acid amounts, multiply the result with the total volume of the semen sample or seminal plasma. Normal citric acid value is 10 mg per ejaculate.
7. Then Cultures were performed in a sterile container; it was placed on a plate with blood agar media.
8. All plates were incubated at 35° C for at least 48 hours.
9. After 48-72 h of incubation, any organisms present on agar were Gram stained and microscopically examined.
10. Then, antimicrobial susceptibility testing were performed by the disc diffusion method.
11. Then, every patient received the proper antimicrobial treatment and then citric acid in semen was measured again.

Ethical considerations:

An approval of the study was obtained from Mansoura University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). P value ≤ 0.05 was considered significant.

RESULTS

The mean age of the studied group was 29.57 ± 4.22 years. The number of male patients with primary infertility was 17 versus 13 with secondary infertility. The number of male patients without systemic disease was 24 versus 6 with systemic disease. The mean pus cell count was 38.00 ± 10.13 million/mL and the number of male patients without urinary symptoms was 19 versus 11 with urinary symptoms. The number of cultures with staph., E. coli, klebsiella, strept. and pseudomonas was 24, 2, 2, 1 and 1 respectively (Table 1).

All studied samples were subjected to antimicrobial sensitivity tests. The most sensitive antibiotics were rifampicin, cefoprazone/sulbactam, amoxicillin/clavulanic acid and piperacillin/ tazobactam. While, the most resistant antibiotics were ciprofloxacin, cephalexin and levofloxacin. Other antimicrobial sensitivity patterns are shown in table (2).

The average citric acid was significantly increased after receiving antibiotic 16.64 ± 2.24 versus 3.32 ± 1.40 mg/ejaculate before treatment (p<0.001) (Table 3).

Table (1): Infertility, systemic diseases, pus cells, urinary symptoms and microbiologic data of the studied group

Variable		n=(30)
Infertility	Primary	17 (56.7%)
	Secondary	13 (43.3%)
Systemic diseases	Absent	24 (80%)
	Present	6 (20%)
	Hypertension	2(6.7%)
	DM	4(13.3%)
Urinary symptoms	Absent	19 (63.3%)
	Present	11 (36.7%)
Pus cells (million/ml)	Mean ± SD	38.00 ± 10.13
Cultures	Staph. aureus	24 (80%)
	E. coli	2 (6.7%)
	Klebsiella spp.	2 (6.7%)
	Streptococci.	1 (3.3%)
	Pseudomonas spp.	1 (3.3%)

Table (2): Antimicrobial susceptibility pattern of all studied samples

	Culture Growth N=30					
	Sensitive		Moderate		Resistant	
	N	%	N	%	N	%
Ciprofloxacin	0	0%	0	0%	28	93.3%
Levofloxacin	0	0%	0	0%	23	76.7%
Amoxicillin/ clavulanic acid	14	46.7%	0	0%	5	16.7%
Ampicillin/ sulbactam	2	6.7%	7	23.3%	11	36.7%
Tetracycline	1	3.3%	0	0%	2	6.7%
Gentamycin	0	0%	0	0%	8	26.7%
Piperacillin/ tazobactam	10	33.3%	2	6.7%	15	50.0%
Rifampicin	23	76.7%	0	0%	6	20%
Cefotaxime	6	20%	3	10%	20	66.7%
Amikacin	9	30%	4	13.3%	14	46.7%
Cefoperazone /sulbactam	14	46.7%	3	10%	12	40%
Cefoprazone	2	6.7%	3	10%	2	6.7%
Ceftriaxone	4	13.3%	6	20%	19	63.3%
Cephalexin	0	0%	1	3.3%	26	86.7%
Cefuroxime	6	20%	1	3.3%	19	63.3%

Table (3): citric acid of the studied group before and after antibiotic

Variable	Before n=30	After n=30	p-value
Citric acid (mg/ejaculate)	3.32 ± 0.40	16.64 ± 2.24	**< 0.001***

DISCUSSION

Infertility means the failure to achieve a clinical pregnancy after 12 months of regular unprotected sexual intercourse. It affects approximately 15% of couples [(1)]. The male factor is the main cause of infertility in 20% of cases and contributes in about 50% [(2)]. There are many etiologies for male factor infertility. Infectious processes contribute to about 15% of such cases [(3)]. Male genital tract infections are difficult to detect as they are asymptomatic in many cases [(4)]. Several patients seeking treatment for impaired fertility are increasing so the diagnosis of "silent" genital tract infections should receive attention as the infection may be linked to asthenozoospermia [(5)].

Citric acid has antioxidant and anti-inflammatory functions in tissues damaged by environmental factors. In addition, it favors the synthesis of glycosaminoglycans in various tissues. Citric acid levels are regulated by testosterone, and like fructose can be elevated in oligozoospermic and azoospermic subjects without a convincing clinical explanation [(11)].

This study was carried out on 30 infertile male patients with pyospermia who were aged between 20 and 60 years old to evaluate effect of genital tract infection on level of citric acid in semen of infertile men. In the current study, the mean age of the investigated patients was 29.57 ± 4.22 years old. Similarly, **Elgozali and Colleagues** [(13)] studied a group of patients with infertility and pyospermia and found that their age range was 22-45 years. On the other hand, the range of age showed a little difference from **Abdulla** and **Bhatt *et al.*** [(14, 15)] (31- 40 years and 25-50 years respectively).

In our study regarding the type of infertility, 17 cases (56.7%) had the primary type, whereas the remaining 13 cases (43.3%) had the secondary type. In accordance to these findings, **Abbas** [(16)] found that 56.3% of infertile patients with pyospermia had primary infertility while 43.8% had secondary type. Moreover, **Merino *et al.*** [(17)] noted that among a sample of 180 infertile patients with pyospermia, primary infertility was 112 (59%) and secondary infertility was 78 (41%). In contrary to our findings, **Elgozali *et al.*** [(13)] found that among a sample of 50 infertile men with pyospermia, 45 men (90%) were primary infertile, while only 5 men (10%) were secondary infertile.

In the current study, systemic comorbidities were present in 6 cases (20%). Diabetes mellitus was the commonest one (4 cases 13.3%), followed by hypertension (2 cases 6.7%). Patients suffering from diabetes mellitus were prone to a higher occurrence of certain infections compared with the general population. Indeed, diabetes is considered a risk factor for urinary and genital tract infections, particularly in the setting of uncontrolled hyperglycemia [(18)]

In the present study, the mean of pus cells was 38.00 ± 10.13 /HPF. **Moubasher *et al.*** [(19)] who found that the mean of pus cells in 25 infertile men with documented pyospermia was 3.6 million/ml. It shows a little difference from **Oliva and Multigner** [(20)] who found that the mean of pus cells of 55 infertile men with documented pyospermia was 4 million/ml.

Regarding microbiological profile of the included cases, bacterial growth was detected in all 30 cases (100%). In another study, out of 120 seminal fluid samples collected from infertile men with pyospermia, 74 (61.66%) of samples revealed positive significant growth of bacteria on culture media, while 46 (38.33%) with no growth [(21)]. In the current study, semen cultures of the investigated patients showed growth of positive isolates as follow; positive staphylococci (80%), escherichia coli (6.7%), klebsiella pneumoniae (6.7%), streptococci (3.3%) and pseudomonas aeruginosa (3.3%). In the same way, the frequency rate of staphylococcal aureus infection was 62.5% in seminal fluid infection in a previous study [(22)]. This result is similar to **Isaiah *et al.*** [(7)] who studied the infectious agents that led to male infertility and also studied the percentage occurrence of bacteropsermia and urogenital-caused infertility in adult married males in Benin metropolis and found among the total cases, 92 (65.7%) showed at least one pathogen. Staphylococcus aureus (28.3%), staphylococcus saprophyticus (13.0%), pseudomonas aeruginosa (6.5%), escherichia coli (19.6%) proteus mirabilis (10.8%) klebsiella spp. (10.8%) and proteus vulgaris (10.8%). In a previous study by **Elgozali and his colleagues** [(13)], thirty-four (68%) seminal fluid specimens of the 50 specimens yielded bacterial growth. The isolated bacteria were staphylococcus aureus (61.8%), followed by escherichia coli (35.3%) and then proteus mirabilis (2.9%). Also, it is similar to **Nasrallah *et al.*** [(23)] who found that the commonest isolated organism was staph. aureus (46.2%). On the other hand, another study reported that pathogens detected in the semen of infertile males were as follows; E coli (26.9%), proteus (25%), staph aureus (15%), streptococci (11.5%), klebsiella (11.5%), and pseudomonas (9.6%) [(24)]. Furthermore, **Sasikumar *et al.*** [(25)] noticed that the dominant isolated bacteria were E. coli (40%), S. aureus (28%), pseudomonas aeruginosa (14%), and proteus mirabilis (8%). Additionally, **Bhatt and his colleagues** [(15)] noticed that the commonest isolates were E. coli (41.9%) followed by S. aureus (17.7%), streptococcus faecalis (11.2%), klebsiella pneumoniae (9.6%), staphylococcus saprophyticus (8%), and pseudomonas aeruginosa (4.8%). Abdulla, [(14)] assessed infertile patients with pyospermia and found that the significant growth of positive isolates were enterococcus faecalis (30%), coagulase positive staphylococci (20%), escherichia coli (13.3%), pseudomonas aeruginosa (10%) and klebsiella pneumoniae (10%). Some studies stated that staphylococcus aureus infection is a non-significant contamination condition [26, 27]. The WHO definition of seminal tract infection does not clearly differentiate between infection, contamination and colonization of the genital tract. Semen that passes through the genital tract is sometimes contaminated with Gram positive skin flora such as commensal species of staphylococci, streptococci, and diphteroids. It is generally accepted that Staphylococcus aureus is regarded as pathogenic and needs immediate management [28].

All studied samples in the current study were subjected to antimicrobial sensitivity tests in vivo. The most sensitive antibiotics were rifampicin, cefoperazone/sulbactam, amoxicillin/clavulanic acid and piperacillin/tazobactam. While the most resistant antibiotics were ciprofloxacin, cephalexin and levofloxacin. **Nasrallah and his colleagues** [23] found that bacteria were highly sensitive to piperacillin/tazobactam, imipenem, meropenem, gentamicin, doxycycline, amikacin, and nitrofurantoin. Another study reported that both gram-positive and gram-negative organisms were sensitive to nitrofurantoin (91.5% and 71.7%, respectively) followed by ampicillin/sulbactam (73.9% and 58.9%, respectively), levofloxacin (56.5% and 71.7%, respectively), and gentamicin (56.5% and 53.8%, respectively) [15]. **Isaiah and his colleagues** [7] found that staphylococcus aureus and Staphylococcus saprophyticus were sensitive to imipenem and vancomycin. While, **Uneke and Ugwuoru** [24] reported that staph aureus was more sensitive to nitrofurantoin and perfloxacin, while it was resistant to ampicillin, penicillin, and chloramphenicol. Moreover, streptococci was sensitive to cotrimoxazole and tetracycline, whereas it was resistant to ampicillin and penicillin. In another study, staph aureus was more sensitive to azithromycin, ofloxacin, and sparfloxacin, while it was resistant to cephalexin and cotrimoxazole [13]**.** While, **Abdullah** [14] reported that staph aureus was sensitive to ciprofloxacin and cephaloridine, while it was resistant to penicillin. Furthermore, enterococci were more sensitive to ciprofloxacin and cephaloridine compared to staph, which was resistant to both penicillin and erythromycin. Generally, there is a large variation in the antimicrobial sensitivity and resistance in the literature. Hence, it is recommended to perform culture and sensitivity for all cases presented with pyospermia, rather than following sensitivity parameters reported in the existing literature.

In the current study, citric acid of the studied group (infertile with infected genital tract) before receiving antibiotic was decreased to 3.32 ± 1.40 mg/ejaculate and significantly increased after antibiotic to 16.64 ± 2.24 mg/ejaculate. Similarly, in a prior study aimed to assess the relationship between chlamydia trachomatis (Ct.) infection of the prostate and the concentration of citric acid in 60 patients aged 16–73 years (mean: 40 years) with chronic prostatitis. The study showed that, a reduction in the concentration of citric acid in the prostatic fluid was observed in 34/60 (56.7%) men with chronic prostatitis, with a small decrease being the most common (22/34 patients – 64.7%). Moderate and high decreases were rarer, each found in 6/34 (17.6%) men [29]**.** Moreover, **Wolff *et al.*** [30] noted a significant decrease in the concentration of citric acid in the semen of infertile men who were C. trachomatis positive. **Van der Kammer *et al.*** [31] determined the concentration of citric acid in the semen of men with chronic prostatitis, acute epididymitis, and inflammation of other adnexitis, and examined urethral swabs for C. trachomatis infection. They found a reduced concentration of citric acid in all the study groups of men, without any significant differences. A difference was evident in comparison with controls. Also, a previous study examined 500 Sudanese infertile males with duration of infertility of 2-15 years in azoospermic patients, 4 to 18 years in oligospermic patients, 3-14 years in asthenozoospermic patients, and 2 to 11 years in patients with abnormal sperm morphology. The control group constituted 100 fertile males with normal spermogram. They found that, seminal citric acid levels were significantly reduced in azoospermic and oligospermic patients [32]**.**

Since seminal citric acid is secreted mainly from the prostate gland, any partial or complete obstruction of the ejaculatory ducts may reduce its level in semen. Abnormal testosterone secretion and accessory glands infection may also lead to reduction of seminal citric acid level [32]**.**

CONCLUSION

It was concluded that, citric acid was significantly increased after receiving antibiotics versus before receiving any genital infection treatments in males.

REFERENCES

1. **Hu C, Lu D, Wu T *et al.* (2019):** Glutathione-S-transferases M1/T1 gene polymorphisms and male infertility risk in Chinese populations: A meta-analysis. Medicine, 98 (6): 14166-73.
2. **Agarwal A, Mulgund A, Hamada A *et al.* (2015):** A unique view on male infertility around the globe. Reprod Biol Endocrinol., 13 (1): 37-43.
3. **Sandoval J, Raburn D, Muasher S (2013):** Leukocytospermia: overview of diagnosis, implications, and management of a controversial finding. Middle East Fertil Soc J., 18(3): 129-34.
4. **Low N, Chersich M, Schmidlin K *et al.* (2011):** Intravaginal practices, bacterial vaginosis, and HIV

infection in women: individual participant data meta-analysis. PLoS Med., 8 (2): 1000416-22.
5. **Villanueva-Diaz C, Flores-Reyes G, Beltran-Zuniga M *et al.* (1999):** Bacteriospermia and male infertility: a method for increasing the sensitivity of semen culture. Int J Fertil Womens Med., 44 (4): 198-203.
6. **Pallett A, Hand K (2010):** Complicated urinary tract infections: practical solutions for the treatment of multiresistant Gram-negative bacteria. J Antimicrob Chemother., 65 (3): 25-33.
7. **Isaiah I, Nche B, Nwagu I *et al.* (2011):** Current studies on bacterospermia the leading cause of male infertility: a protégé and potential threat towards mans extinction. N Am J Med Sci., 3 (12): 562-68.
8. **Moustafa M, Sharma R, Thornton J *et al.* (2004):** Relationship between ROS production, apoptosis and DNA denaturation in spermatozoa from patients examined for infertility. Human Reproduction, 19 (1): 129-38.
9. **Weidner W (1992):** Prostatitis—diagnostic criteria, classification of patients and recommendations for therapeutic trials. Infection, 20 (3): 227-231.
10. **Cooper T, Weidner W, Nieschlag E (1990):** The influence of inflammation of the human male genital tract on secretion of the seminal markers α-glucosidase, glycerophosphocholine, carnitine, fructose and citric acid. Int J Androl., 13 (5): 329-36.
11. **Lozano-Hernández R, Gualdrón J, Camejo M *et al.* (2017):** Markers of accessory glands and seminal parameters in infertile men with overweight and obesity. J Metab Syndr., 6: 1000229-34.
12. **Lewis-Jones D, Aird I, Biljan M *et al.* (1996):** Andrology: Effects of sperm activity on zinc and fructose concentrations in seminal plasma. Hum Reprod., 11 (11): 2465-7.
13. **Elgozali S, Omer A, Adam A (2015):** Pyospermia and bacteriospermia among infertile married men attending fertility centers in Khartoum State, Sudan. Am J Res Commun., 3: 43-9.
14. **Abdulla R (2016):** Bacterial Pathogens associated with Pyospermia among Sudanese Infertile Males. African Journal of Medical Sciences, 1 (6): 1-6.
15. **Bhatt C, Mishra S, Bhatt A *et al.* (2015):** Bacterial pathogens in semen culture and their antibiotic susceptibility pattern in vitro. Int J Biomed Res., 6 (11): 909-14.
16. **Abbas D (2019):** Bacterial Infection in Male Infertility in Al-Anbar Province West Of Iraq. Egypt Acad J Biol Sci., 11 (1): 35-40.
17. **Merino G, Carranza-Lira S, Murrieta S *et al.* (1995):** Bacterial infection and semen characteristics in infertile men. Arch Androl., 35 (1): 43-7.
18. **Soh P, Vidal F, Huyghe E *et al.* (2016):** Urinary and genital infections in patients with diabetes: How to diagnose and how to treat. Diabetes & Metabolism, 42 (1): 16-24.
19. **Moubasher A, Sayed H, Mosaad E *et al.* (2018):** Impact of leukocytospermia on sperm dynamic motility parameters, DNA and chromosomal integrity. Cent European J Urol., 71 (4): 470-73.
20. **Oliva A, Multigner L (2006):** Ketotifen improves sperm motility and sperm morphology in male patients with leukocytospermia and unexplained infertility. Fertility and Sterility, 85 (1): 240-3.
21. **Hussein J, Alabbasy A, Al-Rammahi S *et al.* (2019):** Isolation, Identification and Antibiotic Susceptibility Testing of Bacterial Pathogens causing Seminal Fluid infection in Human Males Admitted to the infertility centers in Najaf Province. Indian J Public Health, 10 (9): 709-14.
22. **Amadi E, Nwofor G, Ogbu O *et al.* (2007):** Resistance of Staphylococcus aureus to commonly used antibiotic obtained from Different sources in Abakaliki. African J Sci., 8 (1): 1728-39.
23. **Nasrallah Y, Anani M, Omar H *et al.* (2018):** Microbiological profiles of semen culture in male infertility. Human Andrology, 8 (2): 34-42.
24. **Uneke C, Ugwuoru C (2010):** Antibiotic susceptibility of urogenital microbial profile of infertile men in South-eastern Nigeria. Andrologia, 42 (4): 268-73.
25. **Leisch M, Jansko B, Zaborsky N *et al.* (2019):** Next generation sequencing in AML—on the way to becoming a new standard for treatment initiation and/or modulation? Cancers, 11 (2): 252-57.
26. **Cunha M, Rugolo L, Lopes C (2006):** Study of virulence factors in coagulase-negative staphylococci isolated from newborns. Memórias do Instituto Oswaldo Cruz., 101 (6): 661-8.
27. **Fritz S, Hogan P, Singh L *et al.* (2014):** Contamination of environmental surfaces with Staphylococcus aureus in households with children infected with methicillin-resistant S aureus. JAMA Pediatrics, 168 (11): 1030-8.
28. **Antal G, Lukehart S, Meheus A (2002):** The endemic treponematoses. Microbes and Infection, 4 (1): 83-94.
29. **Zdrodowska-Stefanow B, Ostaszewska-Puchalska I, Badyda J *et al.* (2006):** The effect of Chlamydia trachomatis infection of the prostate gland on the concentration of citric acid. Arch Immunol Ther Ex., 54 (1): 69-73.
30. **Wolff H, Neubert U, Zebhauser M *et al.* (1991):** Chlamydia trachomatis induces an inflammatory response in the male genital tract and is associated with altered semen quality. Fertility and Sterility, 55 (5): 1017-9.
31. **Von der Kammer H, Scheit K, Weidner W *et al.* (1991):** The evaluation of markers of prostatic function. Urological Research, 19 (6): 343-7.
32. **Abdella A, Omer A, Al-Aabed B (2010):** Biochemical markers in semen and their correlation with fertility hormones and semen quality among Sudanese infertile patients. Afr J Biochem Res., 4 (11): 255-60.