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Original Research Article.

Gram Negative Bacteria and Their Effect on Some Semen Parameters Of Infertile Men Attending Fertility Center at Wad Medani (November 2015-2016)

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ABSTRACT

Objective: This study done in fertility center & Dr AL-Tijani Siddig fertility center, Gezira state, Sudan from April to November 2016 to detect the effect of gram negative bacteria on semen parameters among infertile men.

Material and Methods: Semen samples were collected from men attending infertility clinic. Semen parameters were analyzed based on WHO guidelines. Also, samples was subjected to culture using standard bacteriological techniques cultured in MacConky agar, Blood agar, and Nutrient agar.

Results: A total of 52 samples were collected. A number of 19 (50%) had normal sperm count, 11 (28.9%) had oligozoospermia, and 8 (21.1%) had azoospermia. Abnormal motility 50%, Normal motility 50%, abnormal sperm morphology50%, Normal sperm morphology 50%. The prevalence of bacteriospermia was gram positive bacteria 28 (54%) was the most common organism isolated followed by *E.coli* 9 (17%) and less frequently isolated organism was *Klebsiella 1* (2%). Also nigrosin-eosin and leishman stain technique used to differentiated between pus cell and spermatid, it was showed satisfy result.

Conclusion: This study showed that the gram negative bacteria did not played significant role in male infertility. The presence of asymptomatic bacteriospermia did not correlate with abnormal semen parameters.

Key words: Bacteria, Semen, Fertility.

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INTRODUCTION

Infertility is a situation where a couple does not succeed in achieving pregnancy in spite of unprotected sexual intercourse over a period of 12 months.[1,2] In male, infertility is defined as the inability of the male reproductive cells to produce mature, actively motile and functional spermatozoa in sufficient amount that will ensure fertilization of a re- leased ovum in the fallopian tubes.[3] Global variation has been reported in the incidence of infertility from as low as 1% to 1.5% in Korea and Thailand to as high as 13% - 23% in urban areas of Columbia and rural areas of New Guinea. The prevalence of infertility is particularly high in sub-Saharan African, varying from 20% - 40% in some parts of West Africa. The World Health Organization reveals that male reproductive capacity is deficient in not less than 50% of infertile couples in several countries of the world. In Africa, up to 65% of gynaecological consultations are for infertility.[4,5] More than 90% of male infertility cases are due to low sperm counts, poor sperm quality, or both. The remaining cases of male infertility can be caused by a range of conditions including anatomical problems, hormonal imbalances, and genetic defects; the presence of varicocele, sexual dysfunction, genitourinary infections, urospermia, age and nutrition. Other factors include stress and emotions, endocrine and chromosomal abnormalities, excessive alcohol consumption, environmental factors and non-diagnosable causes.

Sperm abnormalities are a critical factor in male infertility. When sperm failed to become mature it remains at stage of spermatid. This cell seems to be similar to polymorphonuclear leukocyte, both cells when found in semen are called "round cell".

LITERATURE REVIEW

Definition

Semen, known as seminal fluid, is an organic fluid that may contain spermatozoa. It is secreted by the gonads (sexual glands) and other sexual organs of male or hermaphroditic animals and can fertilize female ova. In humans, seminal fluid contains several components besides spermatozoa.

Development of the Sperm

The spermatozoa take over 70 days to develop and are produced solely in the testicles. Individual sperm develop within the testicles from a cell called a spermatogonium. These divide to form spermatocytes, which then develop into spermatids.

Spermatids have tail and the cell gradually acquires the ability to move by beating its tail. The spermatid eventually develops into mature spermatozoa. This process takes about 60 days. Another 10 to 14 days are needed to pass through the ducts of each testicle and the epididymis, before it can leave the body in the semen, during ejaculation.

Composition of Semen

Most of the fluid in semen is made up of secretions from male reproductive organs. Semen contains citric acid, free amino acids, fructose, enzymes, phosphorylcholine, prostaglandin, potassium, and zinc which promote the survival of spermatozoa, and provide a medium through which they can move or "swim".

Normal Semen Sample

Normal semen has certain characteristics. These can be broken down into appearance, volume, smell and so forth.[6]

Appearance of Human Semen

A normal sample has a grey-opalescent appearance. If left open for a while the semen initially clots or coagulates over the first hour and then it becomes liquefied.

Volume

A usual semen volume per ejaculate is around 2 to 3 ml or more. Sperm constitute approximately 10% of semen volume.

Smell

A chlorine smell or fishy odor in semen is normal.

Taste

Semen tastes slightly sweet due to a high content of fructose. The taste of semen tends to change slightly from person to person and may be affected by diet.

pH of Semen

The pH range should be 7.2 to 7.8. This is the normal pH of the body. If the pH is lower than 7.2, it may mean that there is a low sperm counts or malformations in the reproductive tract. If the pH is above 7.8, it may indicate a urinary tract infection.

Sperm Concentration

Normal range 20 million/ml or more & total count of ≥ 40 million.

Motility and Viability

The sperm need to be moving and living in order to achieve successful fertilization. For the man to be fertile at least 50 % of the spermatozoa observed need to be active. The movement of the sperms are in a straight-line one hour after ejaculation.

Morphology or Structure of Sperm

After release, the sperm are matured in the seminiferous tubule to mature oval-shaped spermatozoa and have a chromosomal cap. In the semen some cells show different stages of maturity. Some have tapering heads; pinheads, round heads etc. These are not mature sperms. A semen sample usually contains 50% or more normal sperms.

Round Cells in Semen

Many authors are convinced that the presence of inflammatory cells in semen interferes with the fertilizing ability of the spermatozoa.[7] The differentiation of the `round cells' into either cells of spermatogenic or non-spermatogenic origin has an important clinical relevance.

Rounds Cells of Spermatogenic Origin

Male germ cells originate from initial cells that divide repeatedly to give rise in the male to spermatogonia. Thus, mitotic divisions of some of the primitive spermatogonia result in separate cells that contribute to the maintenance of the stem cell pool. Later, by a final division, some spermatogonia give rise to two cells which begin to increase in volume and to develop a specific chromatin pattern. These cells are the primary spermatocytes and have a chromosome set up of 44 + XY. By the next division, each primary spermatocyte gives rise to two daughter cells (secondary spermatocytes) with a chromatin set-up of 22 + X or 22 + Y per cell, in the first meiotic division. These cells, having two different chromatin set-ups, undergo a second meiotic division giving rise to four cells, the spermatids. Two spermatids have a chromosome set-up of 22 +X and two spermatids a chromosome set-up of 22 +Y. From this stage no more division takes place and each spermatid starts the transformation into a spermatozoon. During the transformation to a fully developed spermatozoon, the nucleus of the spermatid undergoes a gradual condensation of its chromatin .During the first step of this condensation , the spermatid nucleus is usually spherical in shape and still has diameter of >5 mm in early spermatids (Sandoz (1952).[8] The condensation of the chromatin then produce a mature sperm head. (Dym, 1971).[9] Multinuclear spermatids are commonly found in the semen samples and need to be carefully distinguished from neutrophils and lymphocytes. Spermatids that have failed to complete development can be found in semen as multinucleated syncytium-like structures, usually containing three or four round nuclei that are located in the periphery of the cell. According to the presentation of these cells in the smear, these independent nuclei could be confounded with the lobes of the multi-lobated nuclei of neutrophils. This is the most difficult cell to distinguish in semen, as the bridges that connect the lobes of the neutrophils nucleus, and that are absent in multinucleated spermatids, are not always easy to observe on the microscope.

Rounds Cells of Non-Spermatogenic Origin

Epithelial cells, polymorph nuclear granulocytes, macrophages, lymphocytes and various epithelial cells are usually referred to this category. In the present study, attempts have been made to compare the leukocytes found in the semen samples with those appearing in a normal blood smear (Sandoz,1952).[8] Although research about the presence of white blood cells in a semen sample has been done for more than 20 years, the lack of consensus is a recurring theme when it comes to definitions, implications and management of this condition in an individual without a reproductive tract infection. Defined in 1992 by the World Health Organization (WHO) as the presence of at least 1×10^6 leukocytes/mL in a semen sample· Leukocytospermia has been found to be present in both fertile and infertile men with and without evidence of infections of the genital tract (Cooper, 2010).[10] In order to define the etiology of leukocytospermia, people who present with this finding can be divided in two groups; those without and those with a genital tract infection (GTI).

The etiology of leukocytospermia in people without a GTI remains unclear. On the other hand, genital tract infections can affect the urethra, epididymis, testicles, and prostate. Asymptomatic infectious urethritis is a rare condition occurring in only 5–10% of the cases, While epididymitis is always symptomatic, chronic orchitis can occasionally be a silent condition. Interestingly, in 2008 Pasqualotto showed a positive correlation between smoking and the presence of leukocytospermia.

Fructose

Fructose is the largest component of semen. Absence of fructose in semen means there is an obstruction or absence of the vas deferens. Abnormal semen is responsible for about 75% of all cases of male infertility. Unfortunately, in many cases doctors never find out why. The following semen problems are possible:-

- ✓ Low sperm count (low concentration) - the man ejaculates a lower number of sperm, compared to other men.
- ✓ Sperm concentration should be 20 million sperm per milliliter of semen. If the count is under 10 million there is a low sperm concentration (subfertility).
- ✓ No sperm - when the man ejaculates there is no sperm in the semen.
- ✓ Low sperm mobility (motility) - the sperm cannot "swim" as well as it should.
- ✓ Abnormal sperm - perhaps the sperm has an unusual shape, making it more difficult to move and fertilize an egg.
- ✓ Sperm must be the right shape and able to travel rapidly and accurately towards the egg. If the sperm's morphology (structure) and motility (movement) are wrong it is less likely to be able to reach the egg and fertilize it.

Causes of Abnormal Semen

- ✓ Testicular infection
- ✓ Testicular cancer
- ✓ Testicular surgery
- ✓ Overheating the testicles: Frequent saunas, hot tubs, very hot baths, or working in extremely hot environments can raise the temperature of the testicles.
- ✓ Tight clothing may have the same effect on some people.
- ✓ Ejaculation disorders: For some men it may be difficult to ejaculate properly. Men with retrograde ejaculation ejaculate semen into the bladder. If the ejaculatory ducts are blocked or obstructed the man may have a problem ejaculating appropriately.
- ✓ Varicocele: This is a varicose vein in the scrotum that may cause the sperm to overheat.
- ✓ Undescended testicle: One (or both) testicle fails to descend from the abdomen into the scrotum during fetal development. Sperm production is affected because the testicle is not in the scrotum and is at a higher temperature. Healthy sperm need to exist in a slightly lower-than-body temperature. That is why they are in the scrotum, and not inside the body.
- ✓ Hypogonadism: Testosterone deficiency can result in a disorder of the testicles.
- ✓ Genetic abnormality - a man should have an X and Y chromosome. If he has two X chromosomes and one Y chromosome (Klinefelter's syndrome) there will be an abnormal development of the testicles, low testosterone, and a low sperm count (sometimes no sperm at all).
- ✓ Mumps: This viral infection usually affects young children. However, if it occurs after puberty inflammation of the testicles may affect sperm production.
- ✓ Hypospadias: The urethral opening is at the underside of the penis, instead of its tip. This abnormality is usually surgically corrected when the male is a baby. If it is not the sperm may find it harder to get to the female's cervix. Hypospadias occur in about 1 in every 500 newborn boys.
- ✓ Cystic fibrosis: Cystic fibrosis is a chronic disease that affects organs such as the liver, lungs, pancreas, and intestines. It disrupts the body's salt balance, leaving too little salt and water on the outside of cells and causing the thin layer of mucus that usually keeps the lungs free of germs to become thick and sticky. This mucus is difficult to cough out, and it clogs the lungs and airways, leading to infections and damaged lungs. Males with cystic fibrosis commonly have a missing or obstructed vas deferens (tube connecting the testes to the urethra; it carries sperm from the epididymis to the ejaculatory duct and the urethra).
- ✓ Radiotherapy: Radiation therapy can impair sperm production. The severity usually depends on how near to the testicles the radiation was aimed.
- ✓ Some diseases: The following diseases and conditions are sometimes linked to lower fertility in males: Anemia, Cushing's syndrome, Diabetes, Thyroid disease.

Medications

- ✓ Sulfasalazine: This anti-inflammatory drug can significantly lower a man's sperm count. The drug is often prescribed for patients with Crohn's disease or rheumatoid arthritis. Usually this side effect goes away after stopping the medication.
- ✓ Anabolic steroids: Often taken by bodybuilders and athletes; anabolic steroids, especially after long term use can seriously reduce sperm count and mobility.
- ✓ Chemotherapy: Some medicines may significantly reduce sperm count.
- ✓ Illegal drugs: Consumption of marijuana.
- ✓ Bacteriospermia affects the normal fertility process by any of these 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.

Justification

Leukocytes in the male genital tract and seminal fluid play a complex and dynamic role. Leukocytes are found in virtually every ejaculate and function at multiple levels. Presence of high leukocyte counts in seminal fluid is an important indicator of male genital tract infection or inflammation. In many studies, leukocytospermia has been associated with male infertility. Decreased sperm count and motility, as well as increased abnormal sperm morphology and a high frequency of immature germ cells have been reported in men with leukocytospermia. Methodological problems have been found to interfere in differentiating leukocyte from immature germ cells that are present in the seminal fluid. Now a days numerous scientific evidences refer to leukocytes and their products as effective factors on sperm and their functions. This is thus strongly believed that diagnosis of leukocytospermia and its causative agents as well as its treatment play a crucial role in evaluation of infertile couples.

AIMS AND OBJECTIVES

General Objective

- To detect the effect of gram negative bacteria on some semen parameters of infertile men.

Specific Objectives:

- To test the efficiency of nigrosin and leishman for differential between pus and immature.
- To determine the frequency of Gram negative bacteria in semen of infertile men attending fertility center.
- Test the susceptibility of gram negative bacteria isolated from infertile men.
- To study the effect of gram negative bacteria in semen parameters.

MATERIALS AND METHODS

Study Design

Cross sectional laboratory based study.

Study Area

Sudan, Gezira state, fertility center and Dr AL-Tijani Siddig fertility center.

Study Population

Men attending Gezira fertility center and Dr AL-Tijani Siddig fertility center.

Study Duration

This study was conducted during period from April to October 2016.

Sample Size

Semen samples in this study about 52 samples were collected from Gezira fertility center and Dr AL-Tijani Siddig fertility center.

Inclusion Criteria

All male admitted in Gezira fertility center and Dr AL-Tijani siddig fertility center.

Exclusion Criteria

- Healthy male.
- Male with chronic diseases like (thyroid gland disorder and diabetes mellitus).
- Male under antibiotic treatment.
- Male refuse to underling to this study.

Ethical Consideration

Ethical permission to conduct this research was taken from ministry of health and community development and also from fertility centers at, Gezira state. The ethical approval was confirm by the faculty research board, faculty of medical laboratory sciences of , Gezira university .every participant gave his consent . The specimens and information that collected from patients would not be used for any purposes rather than this study. .

METHOD

Collection and Transport of Semen Samples

- Patient was gave a clean, dry, leak proof container and requested him to collect specimen of semen at home following 3-7 days of sexual abstinence.
- Asked the patient to write his name on the container .date and time of collection period of abstinence and to deliver the specimen to the laboratory within 1 hr after collection. During transit to the laboratory the fluid should be kept near as possible to body temperature.

  This best achieved by placing the container inside a plastic bag and transporting it in pocket in the persons clothing.(Cheesbourg M, 2013).[11]

LABORATORY ANALYSIS

Macroscopic Examination

The semen specimen was described for the color and whether it is clear or turbid pH, Assessing liquefaction, Measuring volume and viscosity.

Microscopic Examination

A wet preparation was prepared for assessing microscopic appearance, sperm motility, sperm number, sperm vitality (if the percentage of motile cells is low), and morphology. Staining and assessing smears for sperm morphology and vitality and to differentiate between leukocyte and spermatid Samples will be stained by nigrosin –eosin and Leishman stain.

Procedure for Nigrosin –Eosin Stain

- Have microscope slide and Nigrosin-eosin stain prewarmed to body temperature.
- Pipette a drop of stain onto the end of a slide then pipette small droplet of semen next to the stain.
- Place the edge of another slide into the drops of stain and semen rock that slide back and forth a few times to mix the sperm and stain, then smear the second slide across the surface of the first.
- Dry the slide rapidly by placing it on a warming plate or waving it back and forth in the air.
- Examine using a bright field microscope.

The nigrosin stain produces adark background on which the sperm stand out as lightly colored objects.Normallive sperm exclude the eosin stain and appear white in color, whearas dead sperm (i.e. those with loss of membrane integrity) take up eosin and appear pinkish in color.

Procedure for Leishman Stain

- Cover the semen smear with the leishman stain.
- After one minute add the phosphate buffer solution for two minutes.
- Wash the slide slowly in tap water and dry.

Culturing of the Semen

The specimen inoculated by wire loop in Blood agar, MacConky agar and Nutrient agar and incubated them at 35-37c. (Cheesbrough M, 2013).[11]

Gram Stain

Most bacteria can be differentiated by their Gram reaction due to differences in their cell wall structure. Those organisms are called gram positive which after being stained dark purple with crystal violet are not decolorized by ethanol. Those organisms are called gram negative which after being stained with crystal violet lose their color when treated with ethanol and stain red with neutral red safranin or other counter stain. Required materials Crystal violet stain (basic stain), Iodine solution (mordant), Decolorize (alcohol) and Safranin (counter stain).Method started by spread smear on slid and then clearly labeled , the prepared slide was left to dry and then was fixed the with flame. The fixed smear was flooded with crystal violet stain for 60 seconds, then rapidly washed off the stain with clean tape water and after that it was covered with iodine solution for 60 seconds, washed off with clean tape water. The stained slide was decolorized rapidly with ethanol for 10 seconds, and washed off with water, after that it was Covered with safranin stain for 2 minute, washed off with clean tape water, the slide was air-dried by placing in draining rack. The smear was examined microscopically with 100x.(Cheesbrough M, 2013).[11]

Biochemical Test

- ✓ Indole.
- ✓ Citrate.
- ✓ Urease.
- ✓ KIA.

Identification of Isolates

The biochemical tests was read to identify the isolates.

Antimicrobial Drug Susceptibility Testing

The Kirby-Bauer method of antibiotic susceptibility was used in this study.

Materials

- Nigrosin-Eosin stain
- Leishman stain
- Culture media (Nutrient agar, Blood agar, and MacConky agar)
- Gloves
- Sterile containers
- Petridish
- Autoclave
- Incubator
- Wire loop
- Normal saline solution
- Slides

CULTURING MEDIA

Blood Agar

Forty grams of blood agar base were suspended in 900ml of distilled water, dissolved by boiling, mixed and sterilized by autoclaving at 121°C for 15 minutes, then cooled to about 50°C, defibrinated sheep blood was added aseptically to give final concentration 10%, mixed gently and 15ml of complete medium were poured into each sterile Petri-dish. The poured plates were allowed to solidify at room temperature on flat surface.

MacConkey Agar

Fifty two grams of MacConkey agar were suspended in one liter of distilled water, brought to boil to dissolve the ingredients completely, then sterilized by autoclaving at 121°C for 15 minutes and poured in sterile Petri-dishes in 15ml amount. The poured plates were left to solidify at room temperature on flat surface.

Mueller-Hinton Agar

This medium was supplied by (Conda). It consists of protease peptone, beef infusion solids, starch. Forty grams of the medium were suspended in one liter of distilled water, then brought to boil to dissolve completely, sterilized by autoclaving at 121°C for 15 minutes, then dispended into sterile Petri-dishes in portions of 15ml each. The poured plates were left to solidify at room temperature on leveled surface.

McFarland Turbidity Standard

The standard has the turbidity of suspension of approximately 1.5×108 bacteria/ml. Swabs were purchased or local prepared.

PROCEDURE

Inoculum Preparation

Using direct colony suspension method 3-5 colonies from an overnight pure single Growth were touched with loop. The selected colonies were emulsified into 5 ml sterile physiological saline in the sterile tube. The tube was compared with the 0, 5McFarland turbidity standard, and density was adjusted by adding either excess amount of saline or increased selected colonies.

Inoculum

Within 15 minutes of prepared inoculum, sterile swab was dipped into the inoculum and the excess was removed by pressing and rotating the swab firmly against the side of the tube above the level of the liquid. Then the swab was streaked all over the surface of the medium three times and finally passed around the edge of the agar surface.

Disk Application

Antimicrobial disks, (Bioanalyse, Turkey) were selected to fit each isolated strain. Using a pair of sterile forceps, 8 disks were placed into each plate for gram positive and gram negative.

Incubation

Within 15 minutes of preparation all plates were incubated aerobically at 35° C overnight.

Reading

After overnight incubation, diameter of each zone of inhibition was measured and recorded to the nearest millimeter. The measurements were made with a ruler on the under surface of the plate without opening the lid. Result of reading was interpreted according to NCCLS Zone Diameter Interpretive Standards.

Statistical Analysis

Data collected were analyzed by using the Statistical Package for the Social Science software (SPSS version 20).

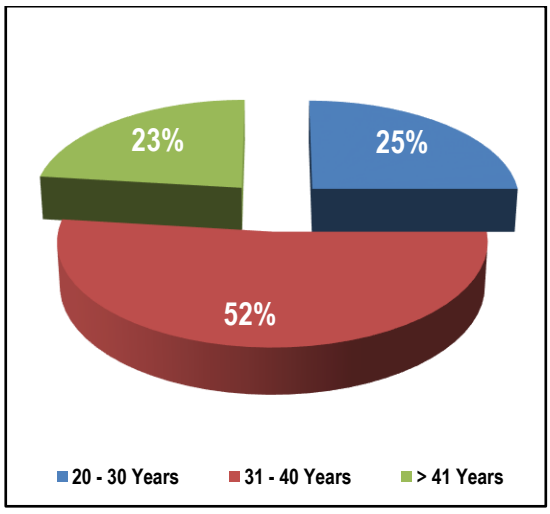

Figure 1: Distribution of the study population according to age groups.

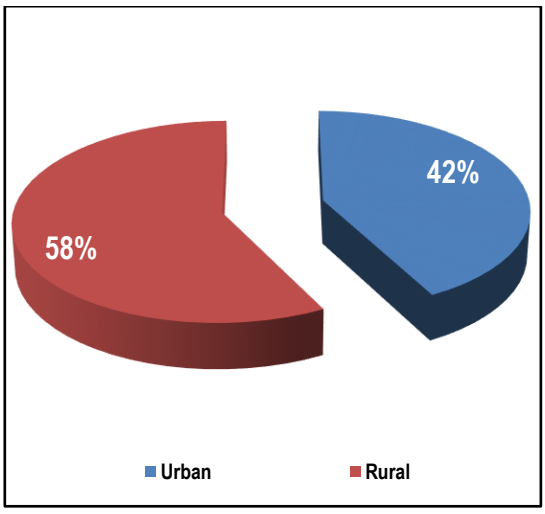

Figure 2: Distribution of the study population according to residence.

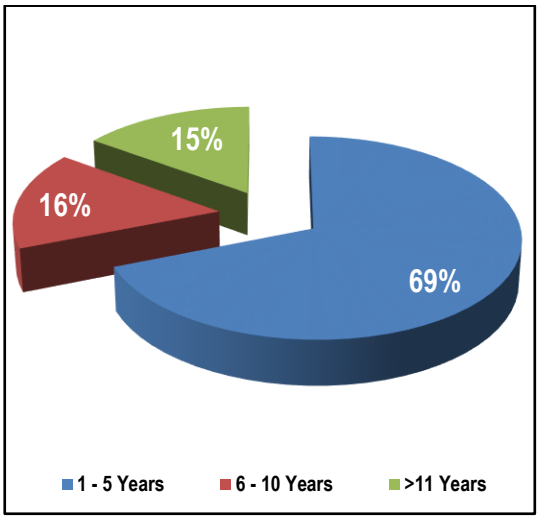

Figure 3: Distribution of the study population according to time of married

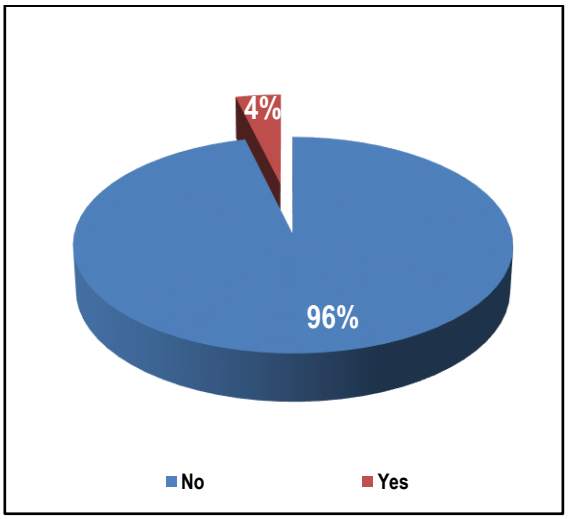

Figure 4: Distribution of the study population according to the history of chronic disease

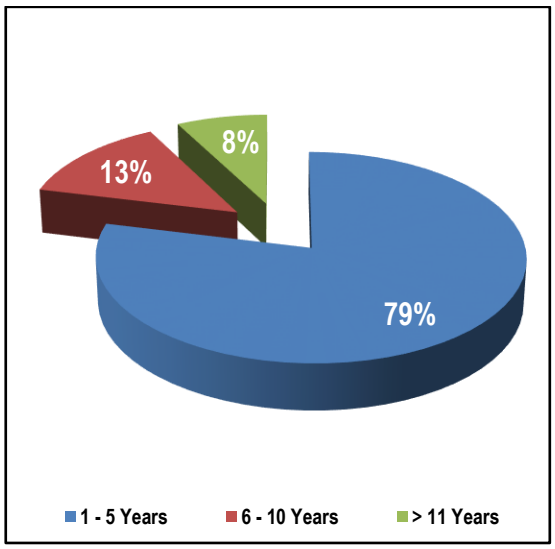

Figure 5: Distribution of the study population according to the duration of infertility

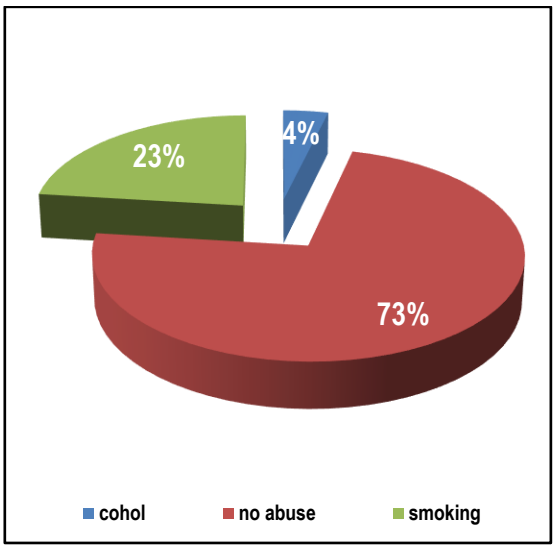

Figure 6: Distribution of the study population according to the abuse.

RESULT AND DISCUSSION

Socio Demographic Characteristic of Study Population

The age groups of the study population were 13 (25%) aged (20-30) years, 27 (51.9%) aged between (31-40) years, 12 (23.1%) aged > 40 years. (Figure 1) 22 (42%) individual come from urban area, 30 (58%) from rural area.

The distribution of study population according to time of married: (1-5years) represent 69%, (6-10 years) represent 16%, and more than 11 years represent 15%.

The distribution of study population according to history of chronic diseases: there are 50 (96%) with no history of chronic diseases, 2 (4%) with history of chronic diseases.

The distribution of study population according to duration of infertility: 41 (1-5 years) represent 79%, 7 (6-10 years) represent 13%, 8 more than 11 years represent 8%.

The distribution of study population according to abuse: there are 2 with alcohol abuse (45), 12 smoking (23%), 38 no abuse (73%).

Frequency and type of bacteria

The distribution of semen samples culture: 45% represent gram positive bacteria, 19% represent gram negative, 27% represent no growth.

The distribution of isolated organism: E.coli 17 %, Klebsiella2%, *Candida* represented 8%, gram positive cocci 54%.

Table 1: Distribution of pus cells among study population: (less than 5 cell/HPF) 50%, (5-10 cell/HPF) 50%.

	Frequency	Percent
Less than 5 /HPF	5	50.0
5-10 /HPF	5	50.0
Total	10	100.0

Table 2: Distribution of spermatids among study population: presented in 40%, absent in 60%.

	Frequency	Percent
Present	4	40.0
Absent	6	60.0
Total	10	100.0

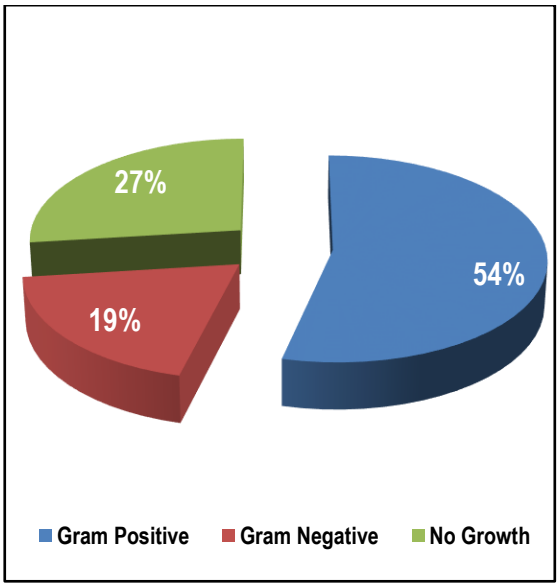

Figure 7: Distribution of semen samples according to gram stain.

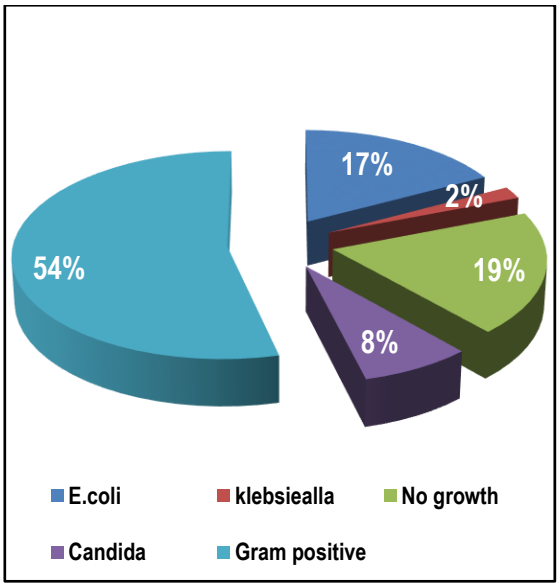

Figure 8: Distribution according to species of bacteria.

Table 3: Susceptibility of isolated bacteria to common used antibiotics

Antibiotic	AS	BA	CF	OF	GM	TZP	AK	CP	CR	LE	TE	CH
Sensitive	6	1	8	8	10	7	6	10	5	10	1	**6**
Resistant	4	9	2	2	0	3	4	0	5	0	9	**4**
Total	10	10	10	10	10	10	10	10	10	10	10	**10**

Table 4: Effect of gram negative bacteria on semen parameters

Parameters	Growth		No growth		
	Frequency	%	Frequency	%	P .value
Count ≥15 millions/ml					
Azoospermia	**8**	**21.1**	**2**	**14.3**	**0.156**
Oligozoospermia	**11**	**28.9**	**1**	**7.1**	
Normozoospermia	**19**	**50**	**11**	**78.6**	
Motility≥40%					
Abnormal motility	**19**	**50**	**5**	**35.7**	**0.359**
Normal motility	**19**	**50**	**9**	**64.3**	
Morphology≥15%					
Abnormal sperm	**19**	**50**	**3**	**21.4**	
Morphology					
Normal sperm morphology	**19**	**50**	**11**	**78.6**	**0.06**

DISCUSSION

Infertility is considered one of the main public health issues, as it affects about 15% of the couples of reproductive age, the male factor is involved in 40% - 50% of infertility cases (Jimoh, et al, 2002).[1] The incidence of infertility in Africa has assumed an alarming proportion as many couples experience this reproductive health problem . This has posed significant inhibition to many reproductive health interventions such as maternal health care, sexually transmitted diseases and HIV/AIDS, as well as created social disharmony within the social structure such as marital instability, infidelity, divorce, emotional and mental stress, violence, denial, stigma and discrimination (CDC,2000).[12] This study was cross-sectional laboratory- based study aimed to identify the common gram negative bacteria associated with infertility and their effects on some semen parameters among infertile men. The study also focused to detect their resistant profile to commonly used antibiotics. The study adopted different standardized tools and methods for the realization of the problem through isolation and identification of bacterial strains which cause infertility. Out of 52 seminal samples examined, 10 of them were found infected with gram negative bacteria. The overall infection rate was 19%. Among the bacterial isolated from the sample, *Escherichia coli* 9 (17.3%) was found to be the most predominant isolate, The same finding was reported by (Ekhaise and Richard, 2008) in Benin City, Nigeria.[13] Other less frequently isolated organisms were Klebsiella pneumoniae 1 (1.9%) agree with the result of research done in India by (Visvanathan R et al, 2016) (6.66%).[14] The nigrosin-eosin stain and leishman stain techniques used to differentiated between pus cell and spermatid showed good result as the following: pus cells (less than 5 cell /HPF) 50%, (5-10 cell/HPF) 50%, similar to previous study done by (Margus Punab et al, 2003) in Estonia.[15] The in- vitro antibiotic

susceptibility pattern of the 10 Gram negative organisms against antibiotic sensitivity discs showed the following: GM, CP, LE were highly active (100%). Similar study conducted by (Mogra et al) reported that E.coli was sensitive to ampicillin, followed by trimethoprim - sulphamethoxaole, erythromycin and chloramphenicol. BA, TE failed to treatment of infection .Inadequate of antimicrobial treatment is an important factor in the emergence of antibiotic resistant bacteria.[16]

Measurement of semen parameters are important indicators for assessment of infertility problems among worldwide e.g: (Vilvanathan R et al, 2015)[14] in India, (Ekhaise et al, 2008) in Nigeria,[13] and another study done to detect reasons and resolve the infertility problem. Data obtained from percentage of types of sperm density revealed Oligospermia (28.9%), Azoospermia (21.1%), and normozoospermia (50%), Abnormal motility 50%. However, Normal motility 50%, abnormal sperm morphology 50%, Normal sperm 50%, this study showed that the gram negative bacteria did not played significant role in male infertility. This study disagree with another study done by (Ekhaise et al, 2008)[13] reported that the presence and profound influence of microorganisms in semen is evidence that microorganisms played significant role in male infertility. Another study implemented by (Visvanathan R et al, 2015)[14] in India showed that there was no definite relationship was established between semen parameters and bacteriospermia and the altered semen quality among different bacterial species also lacks significant association.

CONCLUSION

The result of the current study indicated that bacteriospermia is a common problem associated with infertility in Gezira state.

The result showed that E.coli is the most prevalent gram negative bacteria in this study with incidence of 17%, while Klebsiella with incidence of 2% only, the incidence of all gram negative infection was 19%. Also result showed that the highly active antibiotic were GM, LE, CP. The nigrosin-eosin stain and leishman stain techniques used to differentiated between pus cell and spermatid and showed good result: pus cells (less than 5 cell /HPF) 50%, (5-10 cell/HPF) 50%, similar to previous study done by (Margus Punab et al, 2003) in Estonia.[15]

RECOMMENDATIONS

1. More study with more facilities.
2. Studies with follow up program to see if the drugs resolved the infertility problem and were resisted or not.
3. To control drug resistance, the use of antibiotics should be restricted and be given only after doing culture and sensitivity test.

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