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Studies on bacterial Species in Seminal Fluids and their relationship on Male Fertility and Infertility

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Abstract

To identify bacterial species present in the lower genital tract of males and to investigate the relationship between semin quality and male infertility.The microscopic analysis of(265)semin collected over 24 months from Patient males investigated for infertility, were prospectively assessed.and analyzed The seminal fluids of patients mentioned were investigated laboratory for fertility and both in Hospital and outer clinics. The results shown that from (7) microbial species isolated there are two groups of bacteria II Gram negative: *Escherichia coli* (31) isolates(45.6 %),*Proteus vuligas* (3) isolates (4.4 %) , *Pseudomonas aeruginosa* (3) isolates (4.4%) and group II Gram Positive, *Streptococcus pyogenes* (2) isolates (2.9 %) , *Staphylococcus aureus* (10) isolates (14.7%) and *staphylococcus epidermeds* (3) isolates (4.4%), the infection with microorganisms revealed that it is higher in azoospermic patients than normospermic Patients

Key words: Bacterial Species**,** Seminal Fluid**,** Male Fertility

Introduction

There is difference between the influence of certain microbial infection on male infertility. Several investigators have reported different types of microorganisms in seminal fluid **(Ajabor *et al.*, 1999).** It was reported that detection of bacteria in semin does not essentially suggest infection because bacterial isolates in seminal fluid may signify colonization of the urethral contamination or infection. Enterobacteriaceae, *Chlamydia*, *Ureaplasma* and some gram positive bacteria are the most frequently isolated organisms in industrialized countries **(Keck *et al.*, 1998)**.

In some parts of the world, oligospermia and azoospermia are most common causes of male infertility which has been reported due to bacterial infections**(Megafu, *2007).*** Urinary tract infections are common in men, and clinicians working with infertility frequently encounter patients with these diseases. Infections include either cystourethritis, caused by trivial urinary bacteria or by sexually transmitted

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pathogens affecting fertility. The possible relationship between infection and infertility has been the subject of controversy since the second half of the 1970s2, and several therapeutic trials have been initiated since then. The criteria for infection-associated infertility have been laid down in the World Health Organization (WHO) manuals, and several studies of the pathogenesis of reproductive disturbance in infected men have been published in the past decade ***(*Rowe, 2010*).*** An understanding of the link between infection of the 'accessory sex glands' and reduced male fertility has been scientifically acquired and diagnostic tools are available, but the results of antibiotic treatment in terms of fertility remain disappointing. The last is probably due to the irreversibility of functional damage caused by chronic infection/inflammation. Therefore, prevention, early diagnosis and correct treatment of infections of the male tract, both trivial and sexually transmitted, are of pivotal importance ***(*Sergio *et al.*,***

2007*).* According to (WHO), seminal fluid infection was defined as the presence of significant bacteriospermia (≥10 3 bacteria/ml ejaculate), detection of *Neisseria gonorrhoeae, C. trachomatis, U. urealyticum*; significant leukocytospermia (106 peroxidase positive leukocyte/ml ejaculate). It therefore follows that if some or all the conditions above are not met, the isolation of bacteria in semin are often regarded as contaminants by most practitioners **(Auroux . 2012)).**

Materials

Sampling: (Five hundred seminal fluid specimens from men were investigated for infertility over a period of 9 months. These seminal fluids of patients submitted to the laboratory from the fertility clinics of hospital and outer clinics the practical work was done in The Specialist Medical laboratory". Each specimen was collected by patient himself into sterile bottle. The spatients were instructed on how to collect the specimens and submit to the laboratory within one hour of collection. They were told to first pass urine and then wash their hands and penis with soap, then rinse with water prior to masturbation and ejaculation into sterile container. The semin was collected after the patient had abstained from coitus for at least three days **(Cheesbrough M.1984, 2007)**

Methods: The semen was them cultured on Nutrient, MacConky, Mannitol salt, Thayer martin, Eosin methylene blue, blood, kligler, peptone water, MR-VP, citrate, and chocolate agar media then incubated for 24-48 hours at 37 oC, these media used for *Streptococcus pyogenes, Staphylococcus* aureus, *Staphylococcus epidermidis, Staphylococcus saprophyticus, Eschirishia coli, Proteus mirabilis, Proteus* vulgaris, *Klebseilla pneumoniae, Pseudomonas aeuroginosa, Neisseria*

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gonorrhoeae. Also to confirm the culture results we used Microbial Identification**(Cheesbrough M.1984, 2007)**

The infective microorganisms were identified by Gram stain and cultivation on media for the cultivable microorganisms, others which are not cultivable microorganisms was detected by ELISA technique such as *Chlamydia*, *Ureaplasma* and *Mycoplasma*. The sperm density, volume, viscosity (liquefaction), the percentage of actively motile sperms, the percentage of abnormal forms, the presence or absence of pus cells were assessed. Analysis was carried out immediately after they were received **(Cheesbrough M.1984, 2007)**

The antimicrobial activities of the Purified Products were carried out by using the classical diffusion methods recommended by **kavanagh,(1972)** The antimicrobial activities of the Purified Products were tested by using the classical diffusion methods. Generally this method is based on the observation and measuring of inhibition zones of microbial growth.

Plates are incubated at 37°C for 18-24 hr., and then examined for the presence of zones of inhibition of bacterial growth around antibiotic discs. These are measured by a ruler on the underside of the plate. According to the diameter of the inhibition zone, as read from standard charts for the different antibiotics, it can be determined if the organism is sensitive, moderately sensitive or resistant to the different antibiot

Results:-

Five hundred and seminal fluid specimens from men were investigated for infertility over a period of 9 months. These seminal fluids of patients submitted to the laboratory from the fertility clinics of hospital and outer clinics, the practical work was done and The Specialist Medical laboratory". Each specimen was collected by patient himself into sterile bottle. The subjects were instructed on how to collect the specimens and submit to the laboratory within one hour of collection. They were told to first pass urine and then wash their hands and penis with soap, then rinse with water prior to masturbation and ejaculation into sterile container. The semen was collected after the patient had abstained from coitus for at least three days.and selected (265 Sample From 500) by excluding samples which have the some cultures bacterial Similar Samples . **(Cheesbrough M.1984,2007)**

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Table (1): Full description of patients infected(68 sample) seminal fluid from 265 sample)

Semen EX.. Patient No..	Age	Vol. /ml	Viscosity at 30 ◦C	Total count /ml	Pus/ H.P.F	R.B.Cs / H.P.F	Spermatogenic . Cells / H.P.F	Viability	Abn. Sper m. %
1	21	6	S. viscid	78.000.000	30 – 40	2 - 3	15 – 20	70% A 10% Sl. 20% D	3 - 4
2	32	1	V. viscid	62.000.000	10 – 15	1 – 2	6 - 8	40 % A 20 % Sl. 40 % D	6 - 8
3	22	3	S. Viscid	6.000.000	50 - 60	3 - 4	15 - 20	40 % A. 20 % S. 40 % D.	10-12
7	33	6	L. viscid	60.000.000	15 - 20	2 - 3	1 - 2	70 % A. 10 % S. 20 % D.	5-8
9	25	8	S. viscid	32.000.000	Over 100	2 - 3	4 - 5	60 % A. 10 % S. 30 % D.	10-12
14	21	3	S. viscid	40.000.000	Over 100	3 – 4	1 - 2	50 % A. 20 % S. 30 % D	10-12
15	30	4	S. viscid	40.000.000	Over 100	2 - 3	1 - 2	45 % A. 15 % S. 40 % D.	15-20
27	27	3	S. viscid	38.000.000	20 - 25	2 - 3	5 - 8	50 % A. 20 % S. 30 % D.	12-15
28	31	3	S. viscid	3.000.000	Over 100	3 - 4	6 - 7	40 % A. 20 % S. 40 % D.	8-10
32	40	2	S. viscid	12.000.000	10 - 15	1 - 2	2 - 3	30 % A. 20 % S. 50 % D.	15-20
33	30	3	S.Viscid	40.000.000	10 - 15	1 - 2	2 - 3	50 % A. 20 % S. 30 % D.	12-15
38	22	1	S. Viscid	5.000.000	15 - 20	1 - 2	1 - 2	50 % A. 20 % S. 30 % D.	4-6
40	33	4	S. viscid	52.000.000	50 - 60	2 - 4	2 - 3	60 % A. 20 % S. 20 % D.	12-15
41	34	5	S. viscid	72.000.000	10-12	1 - 2	4 - 5	70 % A. 15 % S. 15 % D.	6-8
45	25	4	S. viscid	62.000.000	10 - 15	3 - 5	4 - 5	70 % A. 10 % S. 20 % D.	5-7
46	25	3	S. viscid	85.000.000	Over 100	2 -3	4 - 6	60 % A. 10 % S. 30 % D.	8-10
47	35	5	S. Viscid	60.000.000	10 - 12	1 - 2	4 - 5	50 % A. 10 % S. 40 % D.	8 - 10
49	40	3	S. viscid	70.000.000	Over 100	2-3	4 - 6	75 % A. 15 % S. 10 % D.	6-8
50	23	5	S. viscid	76.000.000	20 - 25	2 - 4	8 – 10	A. %65 15 % S. 20 % D.	8-10
54	23	1	S. viscid	6.000.000	10 - 15	2 - 4	2 - 3	40 % A. 20 % S. 40 % D.	15-20
55	30	5	S. viscid	82.000.000	20 - 25	3 - 5	1 - 2	80 % A. 10 % S. 10 % D.	4 - 5

60	40	5	S. viscid	32.000.000	30 - 35	2 - 4	15 - 20	50 % A. 20 % S. 30 % D.	8 - 10
67	26	10	S. viscid	16.000.000	15 - 20	2 - 4	4 - 6	40 % A. 20 % S. 40 % D.	10-12
71	30	4	S. Viscid	5.000.000	20-25	2 - 3	10 - 12	40 % A. 20 % S. 40 % D	12-15
72	22	5	S. Viscid	72.000.000	50 - 60	2 - 4	2 - 3	60 % A. 10 % S. 30 % D.	6-8
85	23	6	S. viscid	62.000.000	10 - 15	2 - 3	2 - 3	30 % A. 10 % S. 60 % D.	8 - 10
86	57	4	S. viscid	76.000.000	7 - 8	1 - 2	4 - 5	80 % A. 10 % S. 10 % D.	4 - 6
89	35	4	S. Viscid	60.000.000	Over 100	2 - 3	2 - 3	60 % A. 10 % S. 30 % D.	12-14
91	32	5	S. viscid	56.000.000	Over 100	3 - 4	10 - 15	60 % A. 20 % S. 20 % D.	4 - 5
92	35	3	S. viscid	62.000.000	15- 20	2 - 3	8 - 10	60 % A. 10 % S. 30 % D.	8-10
96	22	2	S. viscid	50.000.000	40 - 50	2 - 3	15 - 20	60 % A. 20 % S. 20 % D.	4 - 5
98	26	3	S. Viscid	52.000.000	Over 100	2-3	10-15	65% A 15% S. 20% D	5-7
100	36	3	V. viscid	76.000.000	10-15	1-2	3-4	70% A. 10%S. 20 %D.	4-6
104	25	2	S. viscid	8.400.000	Over 100	3 - 4	4 - 6	40 % A. 20 % S. 40 % D.	10-12
107	25	2	S. Viscid	60.000.000	Over 100	3 - 4	8 - 10	60 % A. 20 % S. 20 % D.	6-8
128	27	4	S. viscid	75.000.000	15 - 20	3 - 5	3 - 4	78 % A. 10 % S. 12 % D.	4 - 5
129	20	6	S. Viscid	75.000.000	40 - 50	3 - 4	3 - 4	70 % A. 10 % S. 20 % D.	8 - 10
135	28	3	S. Viscid	20.000.000	10 - 15	0 - 1	2 -4	55 % A. 20 % S. 25 % D.	12-15
145	24	1	S. viscid	21.400.000	10 – 15	0 - 1	8 – 10	20 % A. 10 % S. 70 % D.	12-15
156	29	5	S. Viscid	74.000.000	10 – 15	2 – 3	6 – 8	70 % A. 10 % S. 20 % D	5 - 6
157	24	5	S. viscid	70.000.000	20 - 25	3 – 4	6 – 8	60 % A. 10 % S. 30 % D.	10-12
158	19	4	S. viscid	64.000.000	10 – 15	0 – 1	4 – 5	70 % A. 10 % S. 20 % D.	5 – 6
159	21	5	S . viscid	65.000.000	10 – 15	0 – 1	8 – 10	60 % A. 10 % S. 30 % D.	10-12
160	26	5	S. viscid	74.000.000	10 - 15	0 – 1	6 – 8	60 % A. 10 % S. 30 % D.	6 – 8
162	23	5	S. Viscid	94.000.000	10 – 15	0 – 1	6 – 8	80 % A. 10 % S. 10 % D.	6 – 8

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165	26	3	S. Viscid	18.000.000	20 - 25	2 - 3	2 - 3	50 % A. 20% S. 20 % D.	4 - 5
168	31	5	S. viscid	48.000.000	8 – 10	0 - 1	2 - 3	70 % A. 5 % S. 25 % D.	4 - 5
169	26	4	S. Viscid	16.000.000	15 - 20	2 - 3	4 - 5	40 % A. 20 % S. 40 % D.	5 - 6
171	45	4	L. Viscid	10.200.000	10 - 12	2 - 3	15 - 20	40 % A. 20 % S. 40 % D.	6-8
183	20	2	S. Viscid	6.000.000	50 - 60	2 - 3	10 - 12	40 % A. 20 % S. 40 % D.	5 - 6
208	23	4.0	S.Viscid	65.000.000	Over 100	1-2	3-5	60%A. 30% S. 10% D.	8-10
213	27	2.0	S. viscid	62.000.000	10-12	1-2	5-6	60% A. 20%S. 20% D.	3-4
225	30	4.0	S.viscid♀.	66.000.000	15-20	2-3	2-3	60%A. 10%S. 30%D.	10-12
234	33	2.5	S. viscid	44.000.000	20-25	2-3	4-5	60%A. 20%S. 20%D.	10-12
244	23	3	S. Viscid	30.200.000	10 -12	1 – 2	8 – 10	40 % A. 20 % S. 40 % D.	10-12
247	25	5	S. viscid	60.000.000	50 - 60	2 – 3	4 – 6	75 % A. 10 % S. 15 % D.	10 - 12
253	32	3	S. Viscid	40.000.000	15 - 20	1 – 2	15-20	40 % A. 20 % S. 40 % D.	12-15
255	21	5	S. viscid	10.000.000	10-15	0 – 1	20-30	40 % A. 20 % S. 40 % D.	10-12
256	30	5	S. viscid	3.000.000	0ver100	4 – 5	5 – 8	50 % A. 20 % S. 30 % D.	8- 10
257	37	4	S. Viscid	6.000.000	20-25	0 – 1	1 – 2	40 % A. 25 % S. 35 % D.	12-15
258	40	3	S. viscid	1.000.000	15 - 20	2 – 3	1 – 2	30 % A. 20 % S. 50 % D.	12-15
259	25	4	S. viscid	18.000.000	6 – 8	0 – 1	5 – 6	60 % A 20 % S. 20 % D.	8-10
260	30	5	S. viscid	Asospermia	20-25	1 – 2	Zero	Zero	Zero
261	22	6	S. viscid	Asospermia	10-15	0 – 1	0 – 1	Zero .	Zero
262	29	5	S. Viscid	64.000.000	10-12	0 – 1	1 – 2	70 % A. 10 % S. 20 % D.	10-12
263	26	3	S. Viscid	16.000.000	8 –10	1- 2	2 – 3	60 % A. 10 % S. 30 % D.	8-10
264	32	3	S. Viscid	1.000.000	25-30	1 – 2	2 – 3	Zero	10-12
265	37	5	S. Viscid	74.000.000	Over 100	1 - 2	3 – 5	70 % A. 10 % S. 20 % D.	5 – 6

D: Dead (immotile) S. :Semi , A. : Active , S. : Sluggish ,V.: Very : L. : Low

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Examination of seminal fluid 500 sample (age 15-60) and select 265 random sample measurement of volume (0.5 ml – 10.0 ml) different volume according to present seminal fluid and measurement viscosity at 30^{o}C, volume, pH and examination of a wet preparation to estimate the percentage of motil spermatozoa, report type of motility and the percentage of viable forms, look for cells (pus cells, R.B.Cs, spermatogenic cells and bacteria, total count (ml, viability and abnormal sperm count, variable count and examination of stained preparation to estimate the percentage of spermatozoa with normal morphology of spermatozoa under microscopic for detect normal and abnormal spermatozoa.

Results showing that different total count, volume, viscosity at 30^{o}C, pus cells, bacteria deed, RBCs, spermatogenic cells/ H.P.F and different viability (active, sluggish, abnormal forms count and classification according to sperm count/ml through pus cells and total sperm count normal sperm count (40-150 million) moderate (20-40 million), weak (1-20 million) and azoospermia (zero sperm or no sperm and classification according to viability in for orders of sperm count/ml and found variable motility viscosity, volume/ml, total count, pus cells/ml, RBCs/ml, spermatogenic cells, abnormal forms from sample to other and this study represent that bacterial infection of the semen can have a direct role in spermatozoid parameters. Change and may result in men's infertility. In the present study 68 semens (Pur Culture)of bacterial infection were infected by different species bacteria but those infected by these bacteria it may be conducted that the bacteria causing genital tract infection can defect the morphology and the motility of men's spermatozoa.

Table (2): Inhibition Zone of the tested isolates against different antibiotics / (cm .): 68 Pur Culture.

Sample number	Inhibition Zones of Antibiotics in cm											
	CIP	AK	OFX	GM	AMP	DA	E	P	CRO	SAM	FL	azm
1	3.0	2.4	2.8	2.2	1.4	R	R	R	R	R	R	R
2	2.8	2.3	2.0	R	1.2	R	R	R	R	R	R	R
3	2.7	2.9	2	R	1	R	R	R	R	R	R	R
7	2.3	R	2.8	1	R	R	2	R	R	R	R	R
9	1.1	0.5	R	R	R	2.6	0.8	R	R	R	2.2	R
14	2.8	2.6	R	R	R	R	1.2	0.9	R	R	R	R
15	2.4	R	R	R	R	R	2.0	2.2	R	R	R	1.0
27	3.0	2.6	R	R	1.0	R	R	R	R	R	R	R
28	2.6	R	R	R	R	R	R	R	R	R	R	2.0
32	2.4	R	2.9	R	R	R	R	R	1.0	2.0	R	R
33	3.0	2.6	1.4	R	R	R	R	R	R	R	R	R
38	2.8	R	R	R	R	R	1.2	R	R	R	R	2.6

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Sample number	Inhibition Zones of Antibiotics in cm											
	CIP	AK	OFX	GM	AMP	DA	E	P	CRO	SAM	FL	azm
40	R	R	1.4	R	R	R	R	R	3.0	R	R	2.8
41	R	2.2	R	0.9	R	2.8	R	R	R	R	R	R
45	3.0	2.7	R	R	R	R	1.4	R	R	R	R	R
46	2.7	2.2	R	R	R	2.9	1.3	R	R	R	R	R
47	1.6	1.0	R	R	2.6	2.0	2.4	R	R	R	R	R
49	2.8	2.4	R	R	R	R	1.2	R	R	R	R	R
50	2.9	R	R	R	R	R	R	R	3.0	R	R	R
54	R	1.2	3.2	R	R	R	R	R	R	2.3	2.8	R
55	2.9	2.0	R	R	R	R	3.0	R	R	R	R	R
60	3.0	3.2	R	2.6	1.5	R	1.0	R	R	R	R	R
67	2.4	3.0	R	R	R	R	2.6	R	R	R	R	R
71	3.0	1.2	R	R	R	R	2.5	R	R	R	R	R
72	3.0	2.4	R	R	R	R	1.4	R	R	2.0	R	R
85	3.0	R	R	2.6	2.0	R	R	R	R	1.3	R	R
86	3.0	R	1.6	R	R	R	2.8	R	R	R	R	R
89	2.8	R	R	2.6	2.0	R	1.4	R	R	R	R	R
91	2.8	R	1.6	R	R	R	R	R	R	2.3	R	R
92	2.9	R	R	2.0	2.7	1.2	R	R	1.5	R	R	R
96	2.7	R	2.2	R	R	R	1.6	R	R	R	R	R
98	2.8	R	R	2.6	R	R	R	R	2.0	1.3	R	R
100	2.4	2.2	R	R	R	R	R	R	R	1.4	R	R
104	1.8	2.7	R	2.4	R	R	2.0	R	R	R	R	R
107	2.9	R	2.6	R	R	R	2.2	R	R	R	R	R
128	2.6	R	2.0	R	R	R	R	R	R	R	R	R
129	2.8	R	2.6	R	R	R	R	R	2.0	1.8	R	R
135	R	R	2.7	R	1.8	R	R	R	R	R	2.0	R
145	R	R	2.6	R	R	R	R	R	1.0	R	R	R
156	2.7	R	3.0	R	1.2	R	R	R	2.2	R	R	R
157	2.6	R	2.0	R	R	R	1.1	R	R	R	R	R
158	2.0	R	2.4	R	1.0	R	R	R	R	2.6	R	R
159	2.5	R	2.0	R	R	R	R	R	1.6	R	R	R
160	R	2.0	2.7	R	R	R	R	R	2.2	R	R	R
162	2.2	2.0	R	R	R	R	R	R	R	R	R	2.0
165	2.5	R	2.2	R	R	R	R	R	R	R	R	R
168	2.9	R	2.0	R	1.0	R	R	R	0.9	1.2	R	R
169	2.8	1.8	R	R	R	R	R	R	2.2	R	1.4	R
171	2.0	R	1.8	R	2.5	R	R	R	1.4	R	R	R
172	2.3	2.0	R	1.0	R	R	R	R	2.6	R	R	R
183	2.7	2.0	2.3	1.6	R	R	R	R	R	R	R	R
208	3.0	1.6	R	R	R	R	2.9	R	R	R	R	R

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Sample number	Inhibition Zones of Antibiotics in cm											
	CIP	AK	OFX	GM	AMP	DA	E	P	CRO	SAM	FL	azm
213	2.5	R	2.7	R	1.0	R	R	R	R	1.2	R	R
225	R	2.7	R	1.4	R	2.8	R	R	R	R	R	R
234	2.6	R	R	2.2	1.0	R	R	R	R	R	R	R
244	2.2	2.0	R	R	R	R	R	R	R	1.3	R	R
247	2.4	2.5	R	R	R	R	R	0.9	1.8	1.2	2.8	R
253	2.4	2.0	R	R	R	R	1.2	R	R	R	R	R
255	2.8	2.4	R	R	R	R	1.0	R	R	R	R	R
256	2.0	2.2	R	R	2.0	2.6	R	1.0	R	R	R	1.0
257	3.0	R	R	1.0	2.8	2.0	R	R	R	R	R	1.2
258	2.6	R	R	R	2.2	R	2.0	R	R	R	R	1.6
259	2.7	R	R	R	1.2	R	R	R	R	R	R	2.4
260	2.7	2.2	2.4	R	R	R	R	R	R	R	1.0	R
261	2.9	R	R	R	1.6	R	R	R	R	2.0	R	2.4
262	2.8	1.4	R	R	R	R	R	R	R	2.0	R	R
263	2.9	2.2	2.6	R	R	1.3	R	R	R	R	R	R
264	3.0	2.2	R	2.8	1.0	R	0.9	R	R	R	R	R
265	2.7	R	R	R	1.0	R	R	R	2.2	R	R	2.0

*CIP = Ciprocin
*AK = Amikin
* OFX = Ofloxacin
* GM = Gramycin
* AMP = Ampicillin
* DA = Clidamycin
* R = Resistant
* E = Erythromycin
* P = penicillin
*CRO = Ceftriaxone
* SAM = Unasyn
* FL = Flomox
* AZM = Azithromycin

Sensitivity testing must not be performed on commensal organisms or contaminants because this would mislead the clinician and could result in ineffective and unnecessary antimicrobial therapy the causation of side effect and resistance to other potentially pathogenic organisms**(Wood and Shadomy,1983).** Anti-microbial sensitivity (susceptibility) testing is used to select effective antimicrobial drugs.

Sensitivity test was done the tested isolates against different antibiotics for (68) random isolates from 265 sample full description of patients seminal fluid from table (1) which containing pus cells from above (10 H.P.F) in field.

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Pathological Organisms Isolated From Seminal Fluid :

- Identification and percentage of bacteria as etiological agents of seminal tract infection:-

Table (3): Characterization of the etiological agents of seminal tract infection (68) infected Cases from (265) Seminal Fluid :-

Organism	No. of isolates	Percentage of isolates	Fertile	Infertile
1)Gram negative microorganism	31	45.6%	10	21
Escherichia coli	3	4.4%	1	2
Proteus vuligars	3	4.4%	1	2
Pseudomonas aeruginosa				
2)Gram positive microorganism	16	23.5%	7	9
Streptococcus faecalis	2	2.9%	1	1
Streptococcus pyogenes	10	14.7%	3	7
Staphylococcus aureus	3	4.4%	1	2
Staphylococcus epidrmids				

From our study we have the following results :-

1)Gram negative microorganism

1.. ***Escherichia coli***: 31isolates = 45.6 % : Fertile = 10 and infertile= 2

2.Proteus valgaris: 3 Isolates = 4.4 % : Fertile = 1 and infertile =2

3.Pseudomonas aeruginosa :3 isolsates = 4.4 % fertile = 1 and infertile = 2

11)Gram positive microorganism

1.Streptococcus fecalis(Gram negative - rod shape)=16 isolates =23.5% :Fertile =17 and infertile = 9

2. ***Streptococcus pyogenes*** (Beta hymolytic streptococci): 2 : isolates = 2.9 % : fertile = 1 and in fertile = 2

3.Staphylococcus aureus *(coagulas positive*:hemolytic colonies on blood agar) :10 isolates=14.7 %3 Fertile and 7 infertile.

4.Staphylococcus Epedermides (coagulase negative : non hemolytic colonies on blood agar):3 isolates : fertile = 1 and infertile = 2

Table(3) showing number and percentage of etiological agents of seminal fluid infection :

1. Gram negative (rod – shape):

31 isolates (45.6%.) Escherichia coli, 3 isolates (4.4%.) Protus vuligar and 3 isolates (4.4%)Pseudomonas aeruginosa

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2. Gram positive (cocci form):

16 isolates (23.5%)Streptococcus faecalis, 2 isolates (2.9%) Streptococcus pyogenes, 10 isolates (14.7%.) Staphylococcus aureus and 3 isolates (4.4%.) Staphylococcus saprophiticus.

Biochemical Identification criteria for Isolates Count .

(31)were found to be:grame –ve , Rod .aerobes, Positive (+++) to Motility, Indol Production & Ornithine decarboxylation**), -ve to** Urease, -ve tOxidase**:** -ve**,** to Citrate utilization**:** K / A **to** KIA **(**Kligler Iron Agar**):** K / A:LIA**(**Lysine Iron Agar**):** K / K**.** It is likely to be ***Escherichia coli***

A = Acidic .

K = Alkaline . H2S = black .

3 Test Isolates were found to be:-ve Gram reaction , bacilli**,** Aerobes**,** + + + MIO**(**Motility, Indol Production) , Ornithine decarboxylation**),**+ve Urease.-ve Vp**,** +ve Citrate utilization**:**&-ve(veriabl)**,K/A:** KIA **(**Kligler Iron Agar**)** H2S,K/A:LIA **(**Lysine Iron Agar**).** It is likely to be ***Proteus Vulgaris***

3 **Test Isolates were found to be**:

-ve Gram reaction**,** Rod, Aerobes**,** +Ve, -Ve, -Ve, M I O**(**Motility, Indole Production & Ornithine decarboxylation**).**-veUrease,+ve Oxidase**,** -ve Citrate utilization, K / none : KIA **(**Kligler Iron Agar**):** K / none LIA **(**Lysine Iron Agar**):** It is likely tobe ***Pseudomonas aeruginosa*.**

16 Test Isolates were found to be:

+veGram reaction**: ,** Cocci**,**Facultative anaerobes**,** Arranged in chains**,-ve** Coagulase**,** -ve Optichin**.** -ve Bacitracin, +veBile esculin agar,+ve Mannitol salt agar**,**-ve Catalase**.** γ – haemolytic**.** It likely to be is ***Streptococcus faecalis***.

2 Test Isolates were found to be:

+ve Gram reaction,-ve *Cocci* no Stain,non motil,Facultative anaerobes,-ve Oxidase. Arranged in chains of Variable in Length,-ve Coagulase, -ve Urea hydrolysis, -ve Optichin,-ve Citrate utilization,-ve Indol Formation.+veVoges proskauer test ,-veBacitracin,+ve Bile esculin agar, +veMannitol salt agar, -ve Catalase.+ve Haemolysin (Growth petter On Blood Agar.It is likely to be ***Streptococes pyogenes*** .

10 Test Isolates were found to be:

+veGram reaction**,** Cocci, Facultative anaerobes**,**Arranged in"grape-like" clusters**,** +ve Coagulase,-ve Optichin**,**-ve Bacitracin**,**+ve Bile esculin agar**,-ve** Mannitol salt

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agar**,**+veCatalase**,** β – haemolytic**.** It is likely to be ***Staphylococcus aureus***.

3 Test Isolates were found to be:+ve Gram reaction,Cocci, Facultative anaerobes, Arranged in"grape-like" clusters, Capsulated: non capsulated, non motile, non sporing,-veCoagulase,-ve Optichin, -ve Bacitracin,-ve Bile esculin agar, +ve Mannitol salt agar,+ve Catalase, non-haemolytic &non pegmanted.

It is likely to be ***Staphylococcus epidermedis***.

Table (4): Number of Seminal Fluid Infection in relation to Sperm Density and Percentage (500 Sample).Tested

Sperm	Sperm Density ($x10^6$ cell/ml)	No.of patients	No.of Infected	Percentage(%)
Normospermia	>20	209	31	14.8 %
Oligospermia	2-19	145	51	35.2%
SeverOligospermia	<2.0	59	26	44.1%
Azoospermia	NIL	87	66	75.8 %
Total		500	174	34.8%

The current show that: 31(14.8 %) isolate of 209 Patient are Normospermia ($x10^6$ cell/ml) while 51 (35.2 %) isolate of 145 Patient are Oligospermia (2-19 x 10^6 cell/ml), while 26 (44.1 %) isolate of 59 Patient are Sever Oligospermia (<2.0 x 10^6 cell/ml) while 66 (75.8 %) isolate of 87 Patient are Azoospermia, while 147 (34.8 %) isolate Infected of total 500 Patients.

Table (5) Age classification, number of isolates, leucocytes and sperm count /mL

Age (yrs)	No. examined (174)			No.of positive leukocyte			Spermcount ($x10^6$) mean
21-25	16	17	8	11	7	2	32.3
26-30	19	18	7	13	10	4	30.7
30-35	19	19	6	9	12	2	26.6
36-40	20	19	6	12	7	1	22.5

In the current study. 174 Patients with different age categories, with different count of leucocytes (Pus cells) and results Show that increasing age,and increasing Pus cells lead to decreased Sperm count which lead to infertility.

Discussion:-

Male fertility : depends on the presence of healthy sperm and ability of the male reproductive system to effectively ejaculate sperm into the women's vagina. Male infertility : if non or few sperm don't make as fast as they should to reach the egg

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before it dies, or if a blockage in the male reproductive tract prevents sperm from getting out. **Cheesbrough M.1984, 2007)**

Normal sperm count increased and increased viability because decreased bacterial infection increased fertility and moderate sperm count decreased viability because increased bacterial infection and weak sperm count decreased viability and increased dead sperm increased bacterial infection and leading to infertility.

Increasing Pathogenic bacteria, and decreasing motality and sperm density leading to infertility

In my study is based on 100 male subjects (age 20 ± 42 years) divided into two groups

Group (1) (Infertile): this group consisted of 68 maried male for investigation of an infertile marriage for bacteriological study on the basis of one or more of the following conditions during (2008-2015): a. Epididymal tendness. b.Presence of pus cells in seminal fluid. . c. Positive history of urinary tract infection.

Group (2) (Fertile**):** This group consisted of 32 married males of proven fertility without any urinary complaints epidideymal tenderness or pus cells in their semen samples..In we the current study found in 42.9% of the cases, most of the tested strains were sensitive to ampicillin. Azithromycin, nitrofurantoin, erythromycin and Ciprocin.

Also routine semen examination was done for sperm count, motility, morphology and the presence of pus cells and R.B.Cs.,10% of the infertile patients gave no growth on semen culture,While 90% bacteria were isolated,*Staphylococcus albus* which common contaminant from skin and urethral meatus,42.9% bacterial growth organisms isolated were **:***Streptococcus fecalis*, Coagulas positive *streptococci, E.Coli*, And few cases of *proteus, kelbsiella, pseudomonas* and beta hemolytic *streptocci.,Staphylococcus albus*.*Streptococcus fecalis* and *E.coli* were sensitive to :Unasyn(Ampicillin + Sulbactam),Ciprocin, ., Azithromycin, Flumox, Erythromycin and Ceftriaxon All strains of *staphylococcus aureus* resistance against pencillin.

In the current study, result show : showing number and percentage of etiological agents of seminal fluid infection :

1. Gram negative (rod – shape):

31 isolates (45.6%.) Escherichia coli, 3 isolates (4.4%.) *Protus vuligar* and 3 isolates (4.4%)*Pseudomonas aeruginosa*

2. Gram positive (cocci form):

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16 isolates (23.5%)*Streptococcus faecalis*, 2 isolates (2.9%)*Streptococcus pyogenes*, 10 isolates (14.7%.)*Staphylococcus aureus* and 3 isolates (4.4%.)*Staphylococcus epidrmids* However other studies: **(Keck *et al.*, 1998*).*** *Found that* 15% of male infertility is related to genital tract infection ***(*Wang *etal.,2006)*** found that speciesFrom many infectious microorganisms,U.urealyticum is one of the most common

(Radhouane *et al.* 2007) found that Since 1967, the ureaplasmas have been shown as an aetiology of male infertility**, *(*Emokpae *et al*.,2009**)found that first demonstrated a higher frequency of ureaplasmas in the semen of men with unexplained infertility (76%) compared with fertile men (19%)***(Reichart et al.,2001)****found that* used the ELISA assay results demonstrated that genital mycoplasmas and seem to be widespread among infertile male patients.

(Kong *et al.*, 1999*)***found that U. urealyticum were classified into U. parvum and U.urealyticum, respectively, **(Radhouane *et al*.,2007)** found that Most of the previous reported studies have discussed the role of ureaplasmas in male infertility without discriminating between *U. parvum* and *U. urealyticum*., **(Andrade-Rocha, 2013)** **.**found that as shown respectively by the frequency of 13% and 15.2%. These data

are comparable with those reported in previous studies *U. urealyticum* was the most prevalent species of *Ureaplasma* genus detected (9.2%).towards human sperm cell has been demonstrated in vitro**. (Lackner *et al*. 2006).**Found that The influence or the lack of influence of mycoplasmas and ureaplasmas on seminology may come from the capability of bacterial species to attach to spermatozoa and to affect directly via cellular interactions their vitality, motility, morphology, cellular integrity and their molecular structure or the development of protective immunity to genital infection by the host (population sensitivity to microbial agents) or other host factors.

(Radhouane *et al*,. 2007 & Yoshida *et al,.2003)*found that This ratio is lower than reported by in Maiduguri, where *Staphylococcus aureus* was isolated from 62.5% of the seminal fluids. Most practitioners dismiss this infection as more contamination which is assumed to be of no significance. In my study agreement with The **WHO (2006)** ,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 routinely .contaminated with Gram positive cocci such as*Staphylococcus,Streptococcus.*It is generally accepted that *Staphylococcus aureus* which is coagulase positive is regarded as pathogenic and should be treated.

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(Rosemond *et al.* 2006) found that The longer th infection persist, the greater the damage and loss of germ cells. **(Punab *et al.* 2003).**, found that the rate (percent) of infection increases from normospermic to azoospermic males**.(Rodin *et al*,. 2003)**found that The bacterial infection may be partly responsible for male infertility arises from the clinical observation of the patients' male reproductive system It should be noted that presence of Urogenital Tract Infection and inflammation posed a danger to the fertility profile of male patient and should be eradicated by antibiotics and anti-inflammatory treatment.

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