

## Pyospermia and Bacteriospermia among Infertile Married Men attending Fertility Centers in Khartoum State, Sudan

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### Abstract

**Background:** Bacterial infection of the male genital tract has been considered as one of the causes of male infertility for a long time. Different scientific reports pointed at pyospermia as a surrogate marker of these infections. However, other different causes of inflammation may lead to it.

**Study objectives:** The aim of this work was to study the association of pyospermia and bacteriospermia among the infertile men in fertility centres in Khartoum.

**Material and Methods:** Fifty infertile men voluntarily participated in this study. The required demographic data were collected from their medical records. Seminal fluid specimen was aseptically collected from each participant and examined microscopically and by culture for bacteria.

**Results:** Forty five of the infertile men had primary infertility and five men had secondary infertility. Seminal fluid specimens of eight (16%) of the study sample had pyospermia. Thirty four (68%) specimens yielded bacterial growth. The isolated bacteria were *Staphylococcus aureus* (61.8%), followed by *Escherichia coli* (35.3%) and then *Proteus mirabilis* (2.9%). All the bacterial isolates except for *Proteus mirabilis* were sensitive to azithromycin. All *Staphylococcus aureus* isolates were sensitive to ciprofloxacin, sparfloxacin, ofloxacin in addition to azithromycin.

**Conclusion:** The outcome of this study is that the difference between pyospermia among primarily infertile men and secondarily infertile men was not statistically significant. Moreover, pyospermia was not significantly associated with bacterial growth in the seminal fluid and it is not a strong surrogate marker of bacteriospermia. Seminal fluid culture is an important investigation in the aetiological diagnosis of infertility in infertile men regardless of the level of the pus count in the seminal fluid specimens. More studies are needed to elucidate the full dimension of pyospermia and bacteriospermia associated with male infertility.

**Keywords:** Pyospermia, bacteriospermia, infertile married men

{**Citation:** Sayda M Elgozali, Al Fadhil A Omer, Adam A Adam. Pyospermia and bacteriospermia among infertile married men attending fertility centers in Khartoum State, Sudan. American Journal of Research Communication, 2015, 3(2): 43-49} [www.usa-journals.com](http://www.usa-journals.com), ISSN: 2325-4076.

## Introduction

Infertility has been defined by the International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) in the revised glossary of ART terminology as a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (1, 2). Infertility is often assumed to be a problem that only affects women (1, 2, 3). Historically, male factor infertility has largely been neglected, but it has been found that factors affecting men account for about 40% of the causes and semen quality is used as a surrogate measure of male fecundity (3). Infections of the male genital tract may contribute to infertility whether primary or secondary, by adversely affecting sperm function. Pyospermia has been used as reflection and an indicator of such infection (2,4,5). It is defined as the abnormally high pus cell count in the seminal fluid specimen. The simple laboratory quantitatively as 10 or more pus cells per x40 microscopic objective lens (high power field=HPF). Different studies linked significantly pyospermia to bacterial infection of the genital tract (4,5). Sexually transmitted infection as that due to *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Gardenerella vaginalis* can lead to *pyospermia* and commonly contribute to infertility (4). Other non-sexually transmitted bacteria such as *Staphylococcus aureus* and some members of the Enterobacteriaceae group can also lead genital infection and be reflected as pyospermia. Pyospermia can also appear as non-microbial diseases and differentiation between the two is extremely important in choice of the treatment of the patient. Studies addressing pyospermia as a surrogate marker of bacterial infection of the male genital tract and a contributing factor to male infertility has not been well researched in Sudan. Scarcity of the scientific reports created a burning necessity for such a work and the aim of this scientific work is to study the pyospermia among infertile married men attending Fertility Centres in Khartoum by identification of the bacterial pathogens associated with pyospermia and determination of their antibiotic sensitivity to the commonly used antibiotics.

## Materials and Methods

This study was a cross-sectional descriptive study of a non-probability sampling conducted in Banon and Enjab Gynaecology and Fertility Centres in Khartoum. These centres were medical centres established for delivery of assisted fertility and counseling services.

## Data

The Required data were composed of the demographic information collected by a questionnaire and the results of microbiological testing of the seminal fluid specimens.

## Collection of Seminal Fluid Specimens

Some participants collected their seminal fluid specimens by masturbation and others by coitus interruptus after a period of non-ejaculation of three to five days. Each participant collected his specimen into a disposable wide-mouthed sterile dry plastic container. High care was taken to avoid contamination the seminal fluid specimen from the participant or his surrounding environment. The specimens were delivered to the laboratory immediately after ejaculation (less than 30 minutes) with protection from the extremes of temperature and light. In the laboratory

the specimens were labeled and kept warm in an incubator at 37°C for 10 - 30 minutes to allow liquefaction.

From each specimen, 10 µl of each liquefied, thoroughly mixed seminal fluid were mounted on a warm clean glass slide and covered with a standard cover slip. The preparation was screened under high power lens (X40) objective and the pus cell were counted in 10 fields and the average was calculated.

Blood agar, heated blood agar and MacConkey agar were inoculated from each seminal fluid specimen and incubated aerobically at 37°C for 24 hours. CO<sub>2</sub> was added in the incubation of the heated blood agar. The resulting colonies were examined morphologically for size, colour, shape, consistency and convexity. Gram-stained smear from each growth was examined and Gram-positive bacteria were found and further identified by catalase test, coagulase test, DNase test, growth on mannitol salt agar, and aesculin test. Gram-negative bacteria were also found and identified by oxidase test, Kligler iron agar (KIA) test, indole production test, citrate utilization test, urease production test and motility test. Antimicrobial sensitivity testing was done for the isolated bacteria by Kirby-Bauer disc diffusion technique. Using sterile wire loop, 3-5 well isolated colonies of the test bacterial isolate were emulsified in a test tube containing sterile normal saline. In a good light the turbidity of the suspension was matched with the turbidity of Mac Farland standard of barium chloride. Muller Hinton medium was inoculated with the tested organism using a sterile swab. The swab was streaked evenly over the surface of the medium in four directions, and the plate was rotated to ensure even distribution. The appropriate antimicrobial discs were placed using sterile forceps, evenly distributed in the inoculated plate. The test plates were incubated aerobically at 37°C for 24 hours. Then the plates were examined to ensure that the growth was confluent or semi-confluent. The diameter of each zone of inhibition around antibiotic disc was measured in millimetres using a ruler on the plate, and compared to the WHO Standard Inhibition Zone Reference Table. Antibiotics used were cotrimoxazole, cephalexin, ciprofloxacin, sparfloxacin, azithromycin and ofloxacin.

The sterility and the efficiency of the culture media were tested by incubating 5% of plates aerobically overnight at 37°C then checked for growth.

## Results

The study enrolled 50 married men already diagnosed as infertile with age ranging between 22 and 54 years. Forty five (90%) of them had primary infertility and five (10%) of them had secondary infertility.

The pus cell count of seminal fluid specimens was categorized as high count when 10 pus cells or more were counted in the high power microscopic X40 objective lens field (HPF) and as low count when <10/HPF were counted.

Thirty eight seminal fluid specimens out of the 45 specimens of the primary infertile men had pus cell count of <10/HPF and the remaining seven specimens of this group had pus cell count equaled to or more than 10/HPF. Regarding the pus cell count of the five men with secondary infertility, four specimens had pus cell count of <10/HPF and one specimen with count equal to or more than 10/HPF.

**Table (1) showing pus cell count in the seminal fluid specimens of men according to their infertility**

Parameter	Count of pus cells in semen		Total
	<10/HPF	10/HPF or more	
Primarily infertile men	38 (76%)	7 (14%)	45 (90%)
Secondarily infertile	4 (8%)	1 (2%)	5 (10%)
<b>Total</b>	<b>42 (84%)</b>	<b>8 (16%)</b>	<b>50(100%)</b>

Pus cell count in the seminal fluids of primarily infertile men was not statistically different from that of secondarily infertile men ( $p=0.797$ ).

**Table (2) showing Distribution of seminal fluid specimens investigated according to pyospermia and isolated Bacteria**

Pus cell count/HPF	No. of Growth	No. of no Growth	Total
< 10	22 (44%)	20 (40%)	42 (100%)
$\geq 10$	4 (8%)	4 (8%)	8 (100%)
<b>Total</b>	<b>26 (52%)</b>	<b>24 (48%)</b>	<b>50 (100%)</b>

Twenty two seminal fluid specimens out of the 42 specimens with pus cell count of less than 10 /HPF and four specimens out of the eight specimens with pus cell count that equaled to or more than 10 /HPF yielded bacterial growth. Low pus cells count is a poor indicator of bacterial growth. Pus cell counts are not associated with age groups ( $P=0.384$ ).

**Table (3) showing bacterial isolates from culture of seminal fluid specimens**

Bacterial isolates (n=34)	No. of isolates
<i>Staphylococcus aureus</i>	21(61.8%)
<i>E. coli</i>	12 (35.3%)
<i>Proteus mirabilis</i>	1(2.9%)
<b>Total</b>	<b>34 (100%)</b>

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%)

**Table (4) showing sensitivity pattern of the isolated bacteria to antibiotic drugs**

Bacterial Species	Sensitivity of isolated Bacteria to Antibiotics					
	CO	CN	CP	SP	OF	AZ
<i>Staphylococcus aureus</i>	17 (81%)	16 (76.2%)	21 (100%)	21 (100%)	21 (100%)	21 (100%)
<i>Escherichia coli</i>	4 (33.3%)	5 (41.7%)	10 (83.3%)	10 (83.3%)	10 (83.3%)	12(100%)
<i>Proteus mirabilis</i>	0 (0.0%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	0 (0.0%)

**Key of antibiotics:** CO: Co-trimoxazole, CN: Cephalexin, CP: Ciprofloxacin, SP: Sparfloxacin, OF: Ofloxacin and AZ: Azithromycin.

All the bacterial isolates except for *Proteus mirabilis* were sensitive to azithromycin.

All *Staphylococcus aureus* isolates were ciprofloxacin, sparfloxacin, ofloxacin in addition to the already mentioned azithromycin.

## Discussion

Diagnosis of both or one of the couple as infertile is a shocking news to them. As mentioned earlier in this document, male infertility contributes by about 40% to the causes of infertility but is commonly underrated (3). Practically assessing the male infertility is easier than assessing the female infertility. In spite of this there are infertility causes amenable to treatment among them are the bacterial infections (6). In this study, pyospermia was detected in the seminal fluids of 16% of the participants. Sherifa et al in Saudi Arabia reported recently pyospermia in 17.3% of the seminal fluid specimens of the studied infertile men (7) which was close to our finding.

Regarding the type of infertility, pus cell count in the seminal fluids of primarily infertile men was not statistically different from that of secondarily infertile men in this study ( $p=0.797$ ). Infertility of the men in this study was not surely diagnosed to be due to bacterial infections but the findings constituted an association and the high pus cells count was found among few of them (table 1). Khan et al recommended in their study that pyospermia must be considered by physicians as a male infertility factor (8). This can be conceivable if other causes of infertility that lead to pyospermia are also included. Twenty six (52%) seminal fluid specimens of the 50 specimens yielded significant bacterial growth (table 2). This finding casts doubt on the significance of pyospermia alone as an indicator of bacterial infection of the male genital. Isaiah in Nigeria isolated bacteria from seminal fluid specimens of (65.7%) of their infertile men, a result that was close to this the finding in study (9). For more clarification, twenty two seminal fluid specimens out of the 42 specimens with pus cell count of  $<10$  /HPF and four specimens out of the eight specimens with pus cell count of  $\geq 10$ /HPF yielded bacterial growth. High pus cell count can be one of the indicators of bacterial infection but not the sole one. Therefore, the pus cell count alone in seminal fluid does not confirm or rule out bacterial infection. The isolated bacteria in this study were *Staphylococcus aureus* (61.8%), followed by *Escherichia coli*

(35.3%) and then *Proteus mirabilis* (2.9%) (table 3). This result was similar to result by Isaiah et al (9). All the bacterial isolates except for *Proteus mirabilis* were sensitive to azithromycin (table 4). All *Staphylococcus aureus* isolates were sensitive to ciprofloxacin, sparfloxacin, ofloxacin in addition to the already mentioned azithromycin (table). Bacterial infections are known reported causes of infertility (5,7,8). *Escherichia coli*, which is a common urogenital pathogen ranked second to *Staphylococcus aureus* in this study and its immobilization effect on sperm motility had been documented (10, 11). In conclusion of this discussion it was found that the difference between pyospermia among primarily infertile men and secondarily infertile men was not statistically significant. Moreover, pyospermia was not significantly associated with bacterial growth in the seminal fluid and it is not a strong surrogate marker of bacteriospermia. Seminal fluid culture is an important investigation in aetiological diagnosis of infertility in infertile men regardless of the level of the count in seminal fluid specimens. Azithromycin was found to be the best antibacterial drug for treatment of patients with bacteriospermia according to the in vitro test in this study. More studies are needed to elucidate the full dimension of pyospermia associated with male infertility. The limitation of this study was that seminal fluid specimens were not investigated for other bacterial causes of pyospermia such as *Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Gardenerella vaginalis* due to the scarcity of funds.

### Acknowledgement

We are grateful to the staff in the Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Enjab Fertility and Gynaecology Centre (Khartoum), Banon Fertility Centre (Khartoum), and Al Enaya Polyclinic Medical Laboratory (Khartoum) for their great assistance. Our thanks are also due to Dr. Neima for her great help in the clerical work. We extend the acknowledgement to Dr. Noon and Dr. Shima for their great help.

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