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Effect of bacterial infection, food habit and life style on sperm quality of infertile men in Nashik (Maharashtra)

Samidha Patil and Venkat Hamde

Abstract

Semen is considered as the important marker for male fecundity while assessing infertility in men. The role of infection of male accessory glands are very important as they may cause male infertility. About 360 infertile men were evaluated for bacterial infection, along with sperm parameter (motility, volume, concentration and morphology). Microbiological examination of 360 semen culture, out of this obtained 130 bacterial isolates predominantly *Staphylococcus aureus*, *Enterococcus faecalis*, and *E. coli*. Comparative study between bacterial infection and sperm quality shows that there is negative effect on sperm parameter due to bacterial infection.

Along with bacterial infection, change in life style, food habit, tension, addiction, occupation these factors play in development of infertility. From above data (360 infertile men) of semen sample got 60 infertile men. Out of these 22 men were addicted with tobacco, 18 men were with wrong life style, 10 men with hectic occupation, 10 men with stress and tension. 10 men with addiction but normal life style. All above parameter with respect to concentration, motility, morphology of sperm.

Also got 10 infertile men with normal sperm parameter even though having hectic life style and addiction. Proper counseling of patient regarding food habit and life style of infertile men was done. Drastic change was observed in semen parameter after one month by proper counseling of each infertile men.

Keywords: bacteria, *Staphylococcus aureus*, *Enterococcus faecalis*, and *E. coli*, motility, stress, lifestyle, food habit

Introduction

Parenting is the most beautiful experience in the life. It is God gift for all mankind as well as all creatures. Now a days infertility is increased day by day. In our society for every case of infertile couple, female partners are usually blamed because of lots of misconceptions about what a fertile man is. However with improvement in the level education and awareness these days' trends are gradually changing. Many male partners are now visiting infertility clinics to verify their productive status if they are in doubts. Infertility is a major issue faced by the married couples globally. According to world health organization (WHO) 60-80 million of married couples worldwide live under the stress and infertility. There burden of infertility affecting the Indian couples ranges between 3.9% and 16.8% male factor infertility accounts for 40-50% of the cases with about 2% of men having suboptimal semen parameter. On an average male infertility affects 1 in 20 men.

This Study is dedicated to the countless infertile couples from whom we have learnt so much and who still inspire us to strive harder. Semen is considered as the marker for male fecundity while assessing infertility in men. There are several reasons why a male could be suffering from infertility whether primary or secondary. The role of infection of male accessory sex glands is very important or the potential effects that these condition may have on male infertility. Sperm bacterial contamination is quite frequent and could contribute to the deterioration of the sperm quality of infertile men. Since the ejaculate is a mixture of secretions derived from the urogenital tract and the male accessory glands, seminal culture identifies the presence of germs in any section of seminal tract (De Francesco *et al.* 2011) [17]. On the basis of clinical and experimental research it is found that there is association between isolation of bacteria from semen and deterioration of spermatogenesis and

spermatozoa function which can ultimately lead to infertility. The most frequently isolated microorganism in male patient with genital tract infections or semen contaminations is *Escherichia coli*. The negative influence of this species on sperm quality is partially due to this effect on motility and to the impaired acrosomal function, as demonstrated at the ultra-structure level (Diemer *et al.* 2000).

The influence of gram positive uropathogenic bacteria on sperm morphology and function has been poorly investigated until now. Mehta *et al.* (2002) reported that aerobic cocci are present in about 50 % of semen samples of male partners in infertile couples. *Enterococcus faecalis* was isolated from 53% of patient, *Micrococci* from 20% and alpha haemolytic *Streptococci* from 16 % of the infected samples. Increased prevalence of genital tract infections caused by *E. faecalis* is associated with compromised semen quality in terms of sperm quality in terms of sperm concentration and morphology. The presence of *micrococci* and alpha haemolytic *Streptococci* does not appear to exert any detrimental effect on sperm quality. Although no significant depressor effect of *enterococci* on sperm motility was observed, some researchers described, in an *in vitro* study, a negative influence on membrane integrity of human sperm head, neck and mid - piece, probably mediated by hemolysin, a well known virulence factor of *enterococci*.

The genital *Ureplasmas* and *Mycoplasmas* represent a complex and unique group of micro organisms that have been associated with a wide array of infectious disease in adults and infants. There is a lack of conclusive knowledge regarding the pathogenic potential of genital *Ureplasma* and *Mycoplasma*. Among infectious micro organism, *U. Urealyticum* is one of the most frequently found species (Abdulrazzak and Bakr, 2000; Wang *et al.*, 2006a), and its presence was first recognized, in 1954, in the urethral discharge of men with *nongonococcal urethritis* (HGU) (Shepard 1954). It is a commensal organism of the lower genitourinary tract of sexually active men and women. Many investigators have attempted to determine it and these organism are a cause of infertility. Several studies have demonstrated that *U. Urealyticum* and *M. hominis* play on etiologic role in male infertility, with these infections changing parameters of semen such as spermatozoa density and motility (Abdulrazzak and Bakr, 2000; Zeng *et al.*, 2011; Lee *et al.*, 2013, Huang *et al.*; 2014, Zhang *et al.* 2014a) [7, 13]. other researchers have not found any correlation between *U. urealyticum* and *M. hominis* infection and male infertility (Gunyeli *et al.* 2011) La-Vignera *et al.* 2001). In 1999, phylogenetic analysis resulted in the classification of *Urealyticum* spp. into two species *U. parvum* and *U. urealyticum* (Kong *et al.* 1999) however, most studies on male infertility have discussed the role of *Ureaplsmas* without discriminating between two.

M. genitalium was first isolated, in 1980, from the urethral swabs of two symptomatic men with NGU (Tully *et al.*, 1981). As its discovery, *M. genitalium* has been associated with various urethral disease (Tensen, 2006). While its association with NGU is strong and well accepted, the impact of *M. genitalium* in male fertility remains unclear (Taylor Fobinson and Tensen, 2011). Despite an increase in research into the role *Ureplasmas* and *Mycoplasmas* in male infertility, *M. genitalium* and *U. parvum* remain under investigated, and the role of *U. Urealyticum* and *M. hominis* infection in male infertility is controversial.

Some Gram negative bacteria (Enterobacteriaceae such as *E. coli*, *Klebsiella* species, *Proteus*, *Serratia*, *Pseudomonas* species, etc.) and aetiological agents of sexually transmitted disease (*C. trachomatis*, *Ureaplasmas. Urealyticum. Treponema pallidum*, *N. gonorrhoeae*, etc.) are recognized as certain pathogenesis of the prostate (category II, National Institute of Health classification) (Kneger *et al.*, 1999; Nickel *et al.*, 1999) because they show a close association with positive history (past and / or recurrent UTIS, sexually transmitted disease, urogenital congenital anomalies) and / or positive physical examination for urogenital abnormalities (phimosis, hypospadias, cryptorchidism). Instead, some microorganism of the prostate which are occasionally detectable in the urogenital tract are considered by some authors to be non pathogenic likely pathogens, occasional pathogenic (Gram positive germs, such as *Enterococcus* spp. *Staphylococcus aureus* obligate anaerobes) or possible pathogenic (coagulate. Negative germs. Such as *Staphylococcus haemolyticum*, *Staphylococcus epidermidis*, *Mycoplasmas* (Krieger *et al.* 1999; Nickel *et al.* 1999).

The role played by the micro organism responsible for male urogenital infection and their impact on conventional and unconventional sperm parameters, as well as their hypothetical pathogenic mechanisms, have recently been reviewed (La Vignera *et al.*; 2011)

A strong association between inflammation of male reproductive system and infertility has been reported (Comhaire *et al.*, 1999; La Vignera *et al.*; 2011) in particular, semen quality is altered by the inflammatory process through the impaired secretory capacity of the accessory glands, anatomical obstruction, the presence of on unsuitable micro- environment and / or spermatogenic deregulation (Comhaire *et al.* 1999; La Vignera *et al.*; 2011) Several Analyses of genitor Urinary tract inflammation suggest that a resox imbalance in the semen is a particularly important mediator of the cause effect relationship between semen infection and spermatozoon functional deficient (Aifken *et al.*; 1989; Aydemir *et al.*; 2008).

It is believed that ROS overproduction associated with inflammatory reactions is caused by pathological bacterial strains that colonize or infect the reproductive tract (Comhaire *et al.*; 1999; La Vignera *et al.*; 2011).

In a simplistic model, the kinetics of the urogenital tract inflammatory process may be envisaged in several different phases (Fraczek & Kurpisz, 2007). The presence of bacteria and /or leukocytes in semen represents the initial element. Subsequently, ROS overproduction causes an oxidative imbalance and the accumulation of leukocytes is associated with the initiation of phagocytosis. The activation of specific receptors and signal transduction pathways then occurs, generating biologically active substances such as pro-inflammatory cytokines. These substances then modulate pro and anti oxidative system activation and promote a burst and ROS. Another Phase is represented by spermatozoon peroxidative damage. Finally, remnants of the oxidative stress response may persist in the semen for a long period of time after the infectious agent has been eradicated, further damaging spermatozoa.

During the last decades, several studies have investigated the contribution of male factor and especially the role of bacteriospermia in couples infertility. Both symptomatic and asymptomatic bacteriospermia is associated with both acute and chronic inflammation of the genitourinary tract.

Specifically inflammatory mediators, such as cytokines and reactive oxygen species, restrain the normal function of sertoli cells leading to restricted spermatogenesis and unsuccessful acrosome reaction.

According to the findings of the present systematic review, bacteriospermia clearly affects several semen parameters. However, the impact of the various bacteria seems to differ. The clinical symptomatology does not necessarily correlate with the severity of these symptoms, as mild pathogens such as *mycoplasma spp.* may lead to significant alterations. Given these clinicians should perform routine semen cultures when evaluating infertile couples and treat potential infections, despite the lack of substantial evidence for effect of antibiotics on semen parameters.

In this study tried to investigate whole seminal bacterial communities in Nashik city and provided the most comprehensive analysis of the association between bacterial community and semen quality. The study significantly contributes to the current understanding of the etiology of male fertility. Bacterial infection can be treated by effect of antibiotics. This subclinical male infertility can be reversible. Hence this study can be helped to rule out male infertility. May helpful to our society for awareness of bacterial infection. So keeping in mind the entire present scenario, present study aims at isolating aerobic bacterial

pathogens from semen and assesses its correlation with semen parameter in infertile male partner. The study included all males who attended the infertility clinic of the hospital with their mates. Semen samples were assessed macroscopically and microscopically. macroscopically: volume, colour, pH, viscosity, microscopically: concentration, motility, morphology, leukospermia. Lastly semen samples sent for culture to evaluate bacteriospermia. Along with the bacterial infection lifestyle factors are the amendable habits and ways of life that can greatly influence overall health and wellbeing, including fertility. Male infertility is a widespread condition among couples. In about 50% of cases, couple infertility is attributable to the male partner, mainly due to a failure in spermatogenesis. Several studies have investigated associations between semen quality and the presence of lifestyle stressors, these studies provide evidence that semen quality is impaired by psychological stress. There has been increasing evidence on the global decline in human sperm quality over the past few decades. Levine's group performed a systematic, review and meta regression analysis of the current trends in sperm count. DNA fragmentation is an important factor in the aetiology of male infertility of the current trends in sperm count.

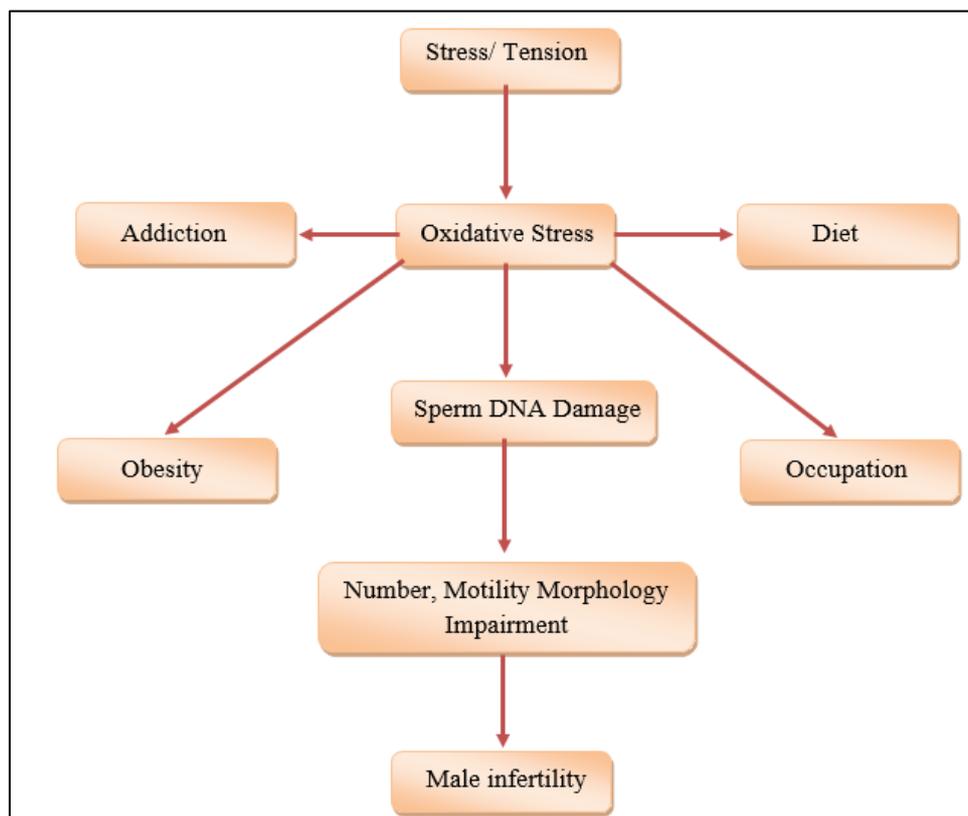


Fig 1: Schematic Representation or different effects of incorrect lifestyle on male fertility

Let us see how life style affects sperm parameter and that cause male infertility

a. Stress /tension: - Stress is a prominent part of any society and infertility itself is stressful due to social pressure, testing, diagnosis, treatments, failures, unfulfilled desires and even economic cost with which it is associated. Stress may reduce luteinizing hormones (LH) and testosterone pulsing, thus reducing in turn spermatogenesis and sperm concentration, quality.

b. Diet: Recently, a review of epidemiological observational studies suggest that diet modification may be useful in modulating male fertility and fecundability.

Some studies shows

- **Healthy diet:** That includes fruits, seafood, poultry, eggs, cereals, leafy vegetables, dark chocolate and low fat dairy product have been positively related to sperm quality.

- However, diets rich in processed meats, fried foods, potatoes, full fat dairy products, caffeine, alcohol and sugar sweetened beverages have been inversely associated with the sperm quality.
- Drinking over 1 litre cola a day might decrease sperm quality by up to 30%
- One study found that following a typical Dutch diet, rich in fruits, vegetables, potatoes, meat, full fat, dairy products, seafood and pastries, increased sperm count by 50%
- Many elements are required for the spermatogenesis and normal functionality of sperm
- Malnutrition or an unhealthy diet can lead to e.g. :-Zinc deficiency, lowering sperm quality

c. Addiction: Cigarette smoking /tobacco smoking is associated with leucocytospermia a major cause of reactive oxygen species (ROS). Increased seminal levels and ROS in smokers expose spermatozoa to oxidative stress, DNA damage, cell apoptosis, aneuploidies and mutation in sperm but also impaired spermatogenesis, sperm mutation.

Alcohol: Alcohol disturbs male reproductive system. It disturbs hypothalamus pituitary –gonadal (HPG) axis. Alcohol appears to interfere with the production of GnRH, FSH, LH and testosterone as well as impair the functionality of leydig and sertoli cells. However, a recent meta-analysis is reported that alcohol intake has a determined effect on semen volume and sperm morphology

d. Obesity: The presence of excess white adipose tissue in obese individual causes increased conversions testosterone to estrogen and affects the HPG axis leading to a reduction in gonadotrophin release. These effects results in secondary hypogonadism and impaired spermatogenesis. Increased production of leptin by the white adipose tissue decreases testosterone production.

e. Occupation: Heavy duties in company, lack of sleep. Exposure to electromagnetic radiation from mobile are associated with reduced sperm motility, concentration.

Men working in various chemical, petrochemical industries may get acquainted through various environment mutagens/agents that are associated with decreased semen quality.

Cadmium causing reduced spermatogenesis and abnormal spermatozoa.

Many pesticides causing decreased semen quality, concentration as well sperm chromosome anomalies

Materials & Methods

In this Study data were collected from infertile men through questionnaires. Semen samples were collected in Nivedita IVF after 3-4 days of sexual abstinence in the aseptic condition in the clean, dry, sterile and leak proof container. Semen sample were produced by masturbation only. Separate room was provided for the collection of semen sample. Written instructions were given for sample collection. 360 semen samples were evaluated. Before collection of semen samples infertile men were examined for urethral discharge, check for varicocele. After collection of semen samples were allowed to liquefy at 37 °C for 30 min. within 5 min 0.5 ml sample was taken for culture. Semen analysis was done by standard criteria of the world health organization 5th edition. Semen sample were assessed macroscopically & microscopically. That is for semen volume, semen colour, pH, viscosity, sperm concentration, sperm motility, sperm morphology, microscopic examination of leukospermia.

Results

360 infertile men were evaluated. Out of that 130 infertile men were bacterial culture positive. According to that we divided into two groups.

Group A- Bacterial culture having normal semen parameter.

Group B- Bacterial culture having abnormal semen parameter.

The semen parameter are summarized in Table 1. There were statistically significant differences in semen parameter among two group Group A and Group B. Motility and count and morphology were higher in group A.

Table 1: Means± standard deviation (SD), of considered variables in group A And group B with contaminated semen samples

Bacterial Species	Patient Semen Quality	Volume in ml	Count Mil/ml	Motility %	Morphology %
<i>E feacalis</i>	Normal Semen	2.33 ±1.05	72.3 ±30.1	51.1 ±9.2	10.5 ±7.73
	Abnormal Semen	1.71 ±0.58	14.4 ±6.11	4.6 ±6.44	2.9 ±0.77
<i>Staphylococcus aureus.</i>	Normal Semen	1.96 ±0.51	56.85 ±21.7	19.2 ±8.20	6.64 ±4.11
	Abnormal Semen	1.84 ±0.77	19.6 ±19.2	5.0 ±10.9	1.95 ±1.36
<i>Eschrichia coli</i>	Normal Semen	3.0 ±1.0	62.33 ±14.00	53.33 ±4.71	5.0 ±1.63
	Abnormal Semen	1.93 ±0.98	77.5 ±171.6	2.5 ±3.35	2.8 ±3.6
<i>Klebsiella pneumoniae</i>	Normal Semen	2.3 ±0.47	66.0 ±10.7	53.33 ±8.49	9.33 ±7.5
	Abnormal Semen	1.77 ±0.70	45.25 ±31.72	2.75 ±1.36	2.75 ±1.63

Table 2: Distribution of frequency and percentage of bacterial isolates having normal semen parameter as well as abnormal semen parameter.

Bacterial Isolates	Normal Semen Parameter		Abnormal Semen Parameter	
	Frequency	Percentage	Frequency	Percentage
<i>Staphylococcus aureus</i>	14	10.7	21	16.1
<i>E. faecalis</i>	22	16.9	15	11.5
<i>E. coli</i>	03	2.3	10	7.6
<i>Kelb pneumoniae</i>	03	2.3	04	3.0
<i>Staphylococcus haemolyticus</i>	05	3.8	01	0.7
<i>Staphylococcus hominis</i>	01	0.7	02	1.5
<i>Acinobacter lwoffii</i>	01	0.7	00	00
<i>Streptococcus agalactiae</i>	04	3.0	05	3.8
<i>Enterococcus cloacae</i>	02	1.5	00	00
<i>Diphtheroids</i>	01	0.7	03	2.3
<i>Sreptococcus sanguins</i>	03	2.3	02	1.5
<i>Streptococcus faecium</i>	03	2.3	03	2.3
<i>Aeromonas salmonicida</i>	01	0.7	01	0.7
Total	63	47.9	67	51.0

All bacterial isolates were indentified and are summarized in Table 2. Most frequently bacterial isolates were identified *Staphylococcus aureus*, *Enterococcus faecalis*, *E. coli*, *Klebsiella pneumoniae*.

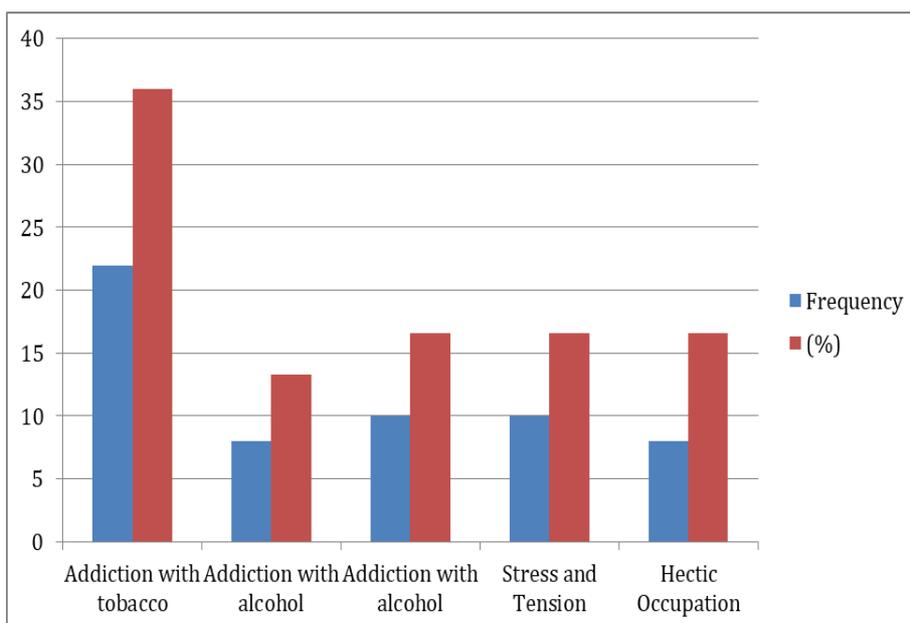
Staphylococcus aureus was found in 26.9%, *E. faecalis* was found in 28.4%, *E. coli* found in 13%, *Klebsiella pneuminiae* was found in 5.3%. Other isolates that is *Staphylococcus haemolytica*, *Staphylococcus hominis*, *Acinobacter lwoffii*, *Streptococcus agalactiae*, *Enterobacter cloacae*, *Diphtheroids*, *Streptococcus sanguins*, *Streptococcus faecium*, *Aeromonas salmonicida* were found in less than 4%.

Results for infertile men having change in life style and food habit

Out of 360 infertile men we separated infertile men having no bacterial infection in semen, but having change in life style, food habit. An overall semen analysis and questionnaires data showed that out of 60 infertile men, 22 men were addicted to tobacco, 10 men were suffered from stress/tension, 10 men were with hectic occupation, 10 men with addiction showed normal semen parameter & 8 men with addiction with alcohol.

Result Chart

Life Style Changes	Frequency	1%	Semen Quality
Addiction with alcohol	8	13.3%	Normal concentration Low motility
Addiction with alcohol	10	16.6%	Normal Concentration
Stress and tension	10	16.6%	Low concentration Low motility
Hectic Occupation	8	16.6%	



Graphical representation of result

I would like to discuss few case studies on male infertility that occurred due to lifestyle changes.

Case 1

- A couple used to come our center having 5-6 yrs of infertility.

- Both partner were evaluated.
- Husband was very busy with his business along with heavy use of mobile phone, addiction of alcohol.
- Initially husband's semen count was normal, but later it declined.
- We guided him, concealed him, he agreed to co-operate.
- After 2-3 month husband's sperm count became normal and his wife conceived with IUI procedure.

Case 2

- Two men were obese, above 100 kg.
- 4-5 years of infertility.
- Female factor was normal. Men having low sperm count.
- Proper counseling was done to obese men, advise for exercise and proper diet was given.
- The men co-operated and weight was reduced by 8-10 kg.
- Drastic changes were seen in their sperm concentration.

All above patient were abnormal semen parameter, with respect to concentration, mobility, morphology. We also got 10 infertile men with normal sperm parameter even though having hectic life style and addiction. Proper counseling was done to each infertile men and repeated semen analysis after 1 month, drastic changes was seen in there semen parameters.

Modification of life style through a structural program and educational environment, nutritional, physical exercise and psychological support combined with the use of nutraceutical antioxidants can prevent infertility and therefore may help couples to obtain better quality of life and optimize their chances of conceptions

Discussion

Many studies have been done to show the genital tract infections and bacteriospermia in male infertility [21]. Microorganism can affect genital tract in many ways, they may agglutinate sperm with head to head or tale to tale, reducing acrosome function, may increase DNA fragmentation due to apoptosis, ROS formation and oxidative stress.

We have taken 10 fertile men having semen positive for bacterial isolates and having normal semen parameter as a control. Another we have taken 10 fertile men having semen negative bacterial isolates and normal semen parameter as a control.

We also found that bacterial isolates present in fertile men having normal semen parameter did not compromise the sperm quality, while bacterial isolates present in infertile men may affect the semen quality. Our findings shows that bacterial isolates positive semen sample may alter the semen quality. Especially motility and morphology significantly decreased in culture positive samples. We also found frequency of *Enterococcus faecalis* increase in normal semen parameter. This can be suggest that there is less concentration of *E. faecalis* or this isolate may considered as normal flora of genital tract.

In this study *Staphylococcus aureus*, *Enterococcus faecalis*, *E. coli*, *Kelbsiella pneumonia* shows negative effect on sperm quality. Mostly they cause adverse effect on motility of sperm and morphology of sperm. The decreased in sperm motility is due to immobilization of sperm or death of sperm

due to bacterial toxin. Immobilization of sperm is may be because of pili or flagella present on some bacterial isolates, they may attached to the mannaos receptor present on spermatozoa. Some other could not find differences between semen characteristics from infected and non infected infertile men. Hillier *et al.* (25) found no differences in semen parameter according to the number of different types of microorganisms. Conversely, in a comparison of 100 infertile and fertile men having a positive semen culture, Jacques *et al.* (24) found a lower percentage of motile spermatozoa [27vs 35% P<0.001].

In conclusion our study suggest that the presence of bacterial isolates may affect the semen quality by the apoptosis and necrosis of spermatozoa cell, which may be responsible for poor motility and morphology of spermatozoa. Infection may cause ROS formation in mitochondria, this cause oxidative stress, apopatsis occur that leads to low motility of spermatozoa. bacterial infection may alter the normal process of spermatogenesis. Keeping in mind male infertility is globally increased, along with andrological diagnostics male genital tract infections are often linked to lower sperm motility and also remain salient or asymptomatic. Appropriate antibiotic therapy can be given to bacterial positive infertile men. This because transmission of the infection to the female parterner must be avoided to increase rate of fertilization to eliminate possible illnesses of the offspring due to infection.

Further study includes *in vitro* cultivation of bacteria. We could see how bacterial isolates affects semen parameters. Also by molecular level investigation we could find out infective pattern of bacterial isolates. Also we could find out why some bacterial isolate were not affect the semen quality, or some were affect semen quality.

References

1. Lewis SEM, Donnelly ET, Sterling ESL *et al.* Nitric oxide synthase and nitrite production in human spermatozoa, evidence that endogenous nitric oxide is beneficial to sperm motility. *Mol hum Repro* 1996;2:873-878.
2. Marconi M, Pilatz A, Wagenlehner F, Diemer T, Weidner W. Impact of infection on the secretary capacity of the male accessory glands *Int Bracj Urol discussion* 2009;35:299-309.
3. Diemer T, Huwe P, Ludwig M, Schroeder-Printzen I, Michelmann HW, Schieter HG *et al.* Inleunce of autogenous leucocytes and *Escherichia coli* on sperm motility parameters *in vivo*, *Andrologia* 2003;35:100-5. doi:10.1046/j 1439-0272.2003.00523.0*. (Pub Med)
4. Gdoura R, Kchaou W, Chaari C, Znazen A, Keskes L, Rebai T *et al.* *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma bominis* and *Mycoplasma genitalium* infections and semen quality of infertile men. *B M C Infect ois* 2007;7:129. doi:10.1186/1471-2334-7-129(PMC free article) (Pub Med)
5. Pellati D, Mylonakis I, Bertoloni G, fiore C, Andrisani A, Ambrosini G *et al.* Genital tract infections and infertility. *Eur J obstet Gynecol Reprod Biol* 2008;140:3-11
Doi:10.1016/jejogrb.2008.03.009.(Pub Med)
6. Review article: Michael Solomon, Ralf Henkel. semen culture and the assessment of genitourinary tract infection. Department of Medical Biosciences,

- University of the Western cape, Bellville, South Africa. year 2017;33(3):188-193.
7. Shun-Long Weng, Chih-Min Chiu, Feng-Mao Lin, Wei-Chih Huang, Chao Liang, Ting Yang *et al.* Bacterial Communities in semen from Men of Infertile Couples: Metagenomic Sequencing Reveals Relationships of Seminal Microbiota to Semen Quality. PLoS One 2014;9(10):e110152. Published online 2014 oct23. Doi: 10.1371/journal.pone.0110152.
 8. Tiwari S. Pattanaik Bacteriological Profile and Seminal Fluid Analysis of Men Attending Infertility Clinic of A Teaching Hospital: Article june-2016.
 9. Joshua S Jue, Tanjith Ramasamy. Significance of positive semen culture in relation to male infertility and the assisted reproductive technology process Tranlational Andrology and Urology. Transl Androl Urol 2017;6(5):916-922. Doi:10.21037/tau.2017.06.23.
 10. Sandro La Vignera, Rosita A Condorelli, Enzo Vicari, Mario Salmeri, Giuseppe Morgia, Vincenzo Favilla *et al.* Microbiological investigation in male infertility: a practice overview. Journal Of Medical Microbiology 2014;63:1-14 Doi 10.1099/jmm0.062968-0
 11. Alo Moses N, Ugah Uchenna I, Elom Emichael O. semen Culture : A Comparative Analysis between Solid Media and liquid Media Supplementation. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) e-ISSN:2278-3008. 2013;5(5):67-72. www.iosrjournals.org
 12. Andrology 3(5) First published : 20 August 2015 <https://doi.org/10.1111/andr.12078> cited by : 22
 13. Huang HL, Zhu KR, Xu SY, Wang LQ, Fan WB, Zhu *et al.* Mycoplasma and ureplasma infection and male infertility : a systematic review and meta-. Wright, Milne, Leeson H, Sperm DNA damage caused by oxidative stress: modifiable clinical, lifestyle & nutritional factors in male infertility. Reprod Biomed Online 2014;28(6):684-703. doi: 10.1016/j.rbmo.2014.02.004 Epub 2014 Mar 4.
 14. Department of Zoology, Centre for Advanced Studies, University of Rajasthan, Jaipur, Rajasthan, India. Impact of age, verified occupation and lifestyle on semen parameter of infertile males in Jaipur: A preliminary study. Asha Asha Year 2014;3(4):278-283. Date of web Publication 16/10/2014
 15. Francesco Marchetti, Andrea Rowan-Carroll, Andrew Williams, Aris Polyzos, Lynn Berndt-Weis M, Carole L. *et al.* Sidestream tobacco smoke is a male germ cell mutagen. Proc Nati Acad Sci U S A. 2011;108(31):12811-12814
 16. Published online 2011 Jul 18. doi:10.1073/pnas. s, 1106896108
 17. Genetics Damayanthi Durairajanayagam Discipline of Physiology, Faculty of Medicine, Sungai Buloh Campu University Teknologi MARA, Selangor Malaysia Semen quality. Life style causes of male infertility Arab J Urol. 2018;16(1):10-20
 18. Published online 2018 Feb 13. doi: 10.1016/j.aju.2017.12.004 teknologi MARA, Selangor Malaysia
 19. Haidl G. Macrophages in semen are indicative of chronic epididymal infection. Arch Androl 1990;25:5-11. doi:10.3109/01485019008987587.
 20. Lewis RW, Harrison RM, Dominguez GJ. Culture of seminal fluid in a fertile clinic Fertil Steril 1981;35:194-198.
 21. McGowan MP, Burger HG, Baker HWG, de Dretser DM, Kovacs G. The incidence of non-specific infection in the semen in fertile and sub-fertile males. Int J Androl 1981;4:657-662.
 22. Jac L, Mathieu D, Auer L, Auroux M. Effect of urogenital infections on sperm parameters and hipofertility in man. Biomed Pharmacother 1980;44:225-228.
 23. Hillier SL, Rabe LK, Muller CH, Zarutskie P, Kusan FB, Stenchever MA *et al.* Relationship of bacteriologic characteristics to semen indices in men attending an infertility clinic. Obstet Gynecol 1990;75:800-804.